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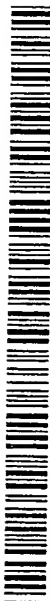
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(54) Title: ANTI-VIRAL AGENTS AND IN-VITRO METHOD FOR THE IDENTIFICATION OF CANDIDATES ABLE TO INHIBIT BINDING OF POLYMERASE TO EPSILON

(57) Abstract: The object of the present invention is to detect and identify a medicament which inhibits activation of viral polymerase via RNA binding, and also to provide an anti-viral agent using a medicament which inhibits activation of viral polymerase via RNA binding. The present invention provides an anti-viral agent comprising, as an active ingredient, a medicament which inhibits activation of viral polymerase via RNA binding.

DESCRIPTION

ANTI-VIRAL AGENTS AND IN-VITRO METHOD FOR THE IDENTIFICATION OF CANDIDATES ABLE TO INHIBIT BINDING OF POLYMERASE TO EPSILON

Technical Field

The present invention relates to an anti-viral agent showing a novel pharmacological action. More specifically, the present invention relates to an anti-viral agent which exerts an inhibitory mechanism of action against activation of viral polymerase via RNA binding. Moreover, the present invention also relates to a method for screening an anti-viral agent by evaluating inhibition of activation of viral polymerase via RNA binding.

Background Art

Hepatitis B virus (HBV) is a causative agent of an acute or chronic hepatitis B. It is estimated that there are more than 300 million HBV carriers throughout the world, and therefore a disease from HBV infection, hepatitis B, is considered to be a serious disease, and has been listed as one of the 10 leading causes of death in the world (World Health Organization warns of growing "crisis of suffering" <http://www.who.int/whr/1997/presse.htm>). Since 1999, the only approved therapy to treat HBV infection had been an administration of interferon, but a nucleoside analogue anti-viral agent, lamivudine, has recently been approved to treat HBV infection, and many anti-viral agents are now being clinically developed. Since HBV can infect and replicate only in humans and chimpanzees, a woodchuck hepatitis virus (WHV) and a duck hepatitis B virus (DHBV), which are belonging to Hepadnaviridae like HBV, are recognized as important models of hepadnavirus infection and are used in studies regarding the replication of hepadnaviruses, including searches for the antiviral agents. The hepadnavirus uses a pregenome RNA (pgRNA), which encodes its own core protein and polymerase (POL), as an intermediate for replication of its own viral DNA by RNA-dependent DNA polymerase activity of the POL (Cell, Summers and Mason, Vol. 29, 403-415 (1982)). The POL recognizes and binds to a ϵ motif, a secondary

structure of RNA which locates near the 5'-terminus on a pgRNA, and then viral core particles are formed by encapsidation with core proteins (The EMBO J., Bartenschlager and Schaller, Vol.11, No.9, 3413-3420 (1992)). Moreover, it is shown that POL binding to ϵ motif act as a protein primer, a starter for viral gene replication, and that the POL covalently bonds to the negative strand of the viral DNA (Cell, Wang and Seeger, Vol.71, 663-670 (1992)). In this experiment system, DHBV POL is expressed by an in vitro translation system using a rabbit reticulocyte lysate, and then deoxynucleoside triphosphates (dNTPs) are added to a POL- ϵ RNA complex in the solution to perform priming reaction, followed by detection of POL covalently bonded to labeled dNTP. Applying this experiment system, anti-viral activity of triphosphorylated nucleoside analogues to compete with dNTP was investigated by using the existing anti-viral agents, and competitive inhibition against each nucleotide had been shown (Journal of Virology, Staschke and Colacino, Vol.68, No.12, 8265-8269 (1994); Antimicrobial Agents and Chemotherapy, Seifer et al., Vol.42, No.12, 3200-3208). Furthermore, a recent study suggests that POL is activated by binding to the ϵ motif on its pgRNA (Journal of Virology, Tavis et al., Vol.72, No.5, 5789-5796 (1998); and Journal of Virology, Xingtai Wang and Jianming Hu, Vol.76, No.12, 5857-5865 (2002)).

However, any compound that inhibits the activation of POL caused by binding to the ϵ motif of pgRNA has not been reported until now.

Disclosure of the Invention

An object of the present invention is to search for and identify a medicament which inhibits activation of viral polymerase via RNA binding. Another object of the present invention is to provide an anti-viral agent using a medicament which inhibits activation of viral polymerase via RNA binding. A further object of the present invention is to provide a method for screening a medicament which inhibits activation of viral polymerase via RNA binding.

In the conventional experiment system, a test medicament is added after

formation of a POL- ϵ RNA complex to analyze competitive action with dNTP. Through intensive studies directed toward the above object, the present inventors have improved the conventional experiment system by providing separately each of POL, ϵ RNA and adding a test medicament before formation of a POL- ϵ RNA complex, and thereby succeeded to find an inhibitory activity of a compound which did not show the inhibitory activity in the conventional experiment system. The present inventors also succeeded in identifying a novel inhibitory mechanism of viral polymerase, thereby completing the present invention.

According to the present invention, there is provided an anti-viral agent comprising, as an active ingredient, a medicament which inhibits activation of viral polymerase via RNA binding.

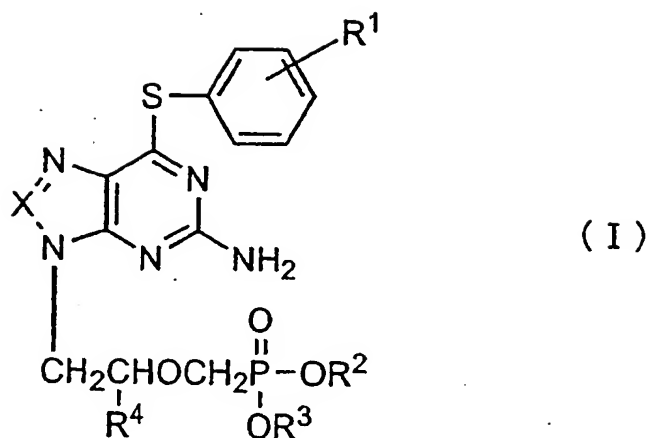
Preferably, activation of viral polymerase via RNA binding is activation of viral polymerase via binding of virus RNA and viral polymerase.

Preferably, RNA is ϵ RNA

Preferably, a pharmacological efficacy is sustained after withdrawing the agent.

Preferably, the virus belongs to Hepadnaviridae, and particularly preferably the virus is a hepatitis B virus.

According to further another preferred embodiment, there is provided the anti-viral agent wherein the medicament is a phosphonate nucleotide compound represented by the following formula (I) or a salt thereof, or a hydrate or solvate thereof:



wherein,

R^1 is a hydroxyl group or a C_1 - C_6 alkoxy group; each of R^2 and R^3 is independently a hydrogen atom, a C_1 - C_{22} alkyl group, an acyloxymethyl group, an acylthioethyl group, or an ethyl group substituted by one or more halogen atoms; R^4 is a hydrogen atom, a C_1 - C_4 alkyl group, a C_1 - C_4 hydroxyalkyl group, or a C_1 - C_4 alkyl group substituted by one or more halogen atoms; and X is CH or a nitrogen atom.

Preferably, R^1 is a hydroxyl group or a methoxy group.

Preferably, each of R^2 and R^3 is independently a hydrogen atom, a C_1 - C_{22} alkyl group, or an ethyl group substituted by one or more halogen atoms.

Preferably, each of R^2 and R^3 is independently a hydrogen atom or a 2,2,2-trifluoroethyl group.

Preferably, R^4 is a hydrogen atom or a methyl group.

Preferably, X is CH.

Particularly preferably, R^1 is a hydroxyl group or a methoxy group, each of R^2

and R^3 is independently a hydrogen atom or a 2,2,2-trifluoroethyl group, R^4 is a hydrogen atom, and X is CH.

Particularly preferably, the medicament is 2-amino-6-(4-methoxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine bis(2,2,2-trifluoroethyl) ester, 2-amino-6-(4-methoxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]-purine, 2-amino-6-(4-hydroxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]-purine, or 2-amino-6-(4-hydroxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine (2,2,2-trifluoroethyl) ester.

According to another aspect of the present invention, there is provided a method for screening an anti-viral agent, which comprises a step of evaluating activation of viral polymerase via RNA binding.

Preferably, the step of evaluating activation of viral polymerase via RNA binding is that of evaluating the degree of binding of virus RNA and viral polymerase.

Preferably, the method for screening an anti-viral agent comprises steps of adding a test medicament to viral polymerase, adding virus RNA, and evaluating the degree of binding of virus RNA and viral polymerase.

Preferably, the method for screening an anti-viral agent comprises steps of adding a test medicament to viral polymerase, adding virus RNA, and judging that the test medicament is an anti-viral agent when the test medicament inhibits the binding of virus RNA and viral polymerase.

Preferably, RNA is ϵ RNA.

Preferably, the virus belongs to Hepadnaviridae.

Preferably, the virus is a hepatitis B virus.

According to further another aspect of the present invention, there is provided an anti-viral agent obtained by the method for screening an anti-viral agent according to the present invention.

According to further another aspect of the present invention, there is provided an anti-viral agent obtained by the steps of conducting the method for screening an

anti-viral agent according to the present invention to obtain an anti-viral substance, producing the thus obtained anti-viral substance by chemical synthesis, and mixing the anti-viral substance with a pharmaceutically acceptable carrier.

According to another aspect of the present invention, there is provided a method for inhibiting a virus, which comprises administration of a pharmacologically effective amount of medicament inhibiting a process where viral polymerase is activated via binding of virus RNA and viral polymerase, to mammals including a human.

According to further aspect of the present invention, there is provided the use of a medicament inhibiting a process where viral polymerase is activated via binding of virus RNA and viral polymerase, for the production of an anti-viral agent.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 shows a schematic diagram of a plasmid construct. In Figure 1, A shows DHBV POL3' ϵ used in the conventional method, B shows DHBV POL ϵ (-) used in the improved method for the preparation of POL, C shows DHBV 5' ϵ used in the improved method as ϵ RNA, D shows DHBV HIS-TP used in the improved method for the preparation of Terminal Protein (TP), and E shows DHBV Δ TP-POL ϵ (-) used in the improved method for the preparation of TP-deleted POL.

Figure 2 shows test results obtained by the conventional method.

Figure 3 shows test results in respect of RNA concentration dependency by an improved method.

Figure 4 shows results of ϵ RNA competition tests obtained by an improved method.

Figure 5 shows test results in respect of the analysis of POL level dependency by an improved method.

Figure 6 shows results of study of the TP or ϵ RNA concentration dependency.

Figure 7 shows results of inhibitory activity of medicament against TP as

protein primer during the formation of TP- Δ TP POL- ϵ RNA complex.

Best Mode for Carrying out the Invention

The anti-viral agent of the present invention is characterized in that it comprises, as an active ingredient, a medicament which inhibits a process where viral polymerase is activated via binding of virus RNA and viral polymerase, and preferably that the pharmacological efficacy is sustained after withdrawing the agent.

Specific examples of medicaments include the phosphonate nucleotide compound of the above formula (I) or a salt thereof, or a hydrate or solvate thereof, and the like.

In the above formula (I), examples of a C₁-C₆ alkoxy group represented by R¹ include a methoxy group, an ethoxy group, an n-propyloxy group, an isopropyloxy group, an n-butyloxy group, an isobutyloxy group, a sec-butyloxy group, a tert-butyloxy group, a pentyloxy group, a hexyloxy group, and the like.

In the phosphonate nucleotide compound of the above formula (I), examples of a C₁-C₂₂ alkyl group represented by R² and R³ include a methyl group, an ethyl group, an n-propyl group, an isopropyl group, an n-butyl group, an isobutyl group, a sec-butyl group, a tert-butyl group, a pentyl group, a hexyl group, a heptyl group, an octyl group, a nonyl group, a decyl group, an undecyl group, a dodecyl group, a tridecyl group, a tetradecyl group, a pentadecyl group, a hexadecyl group, a heptadecyl group, an octadecyl group, a nonadecyl group, an icosyl group, a hencosyl group, a docosyl group, and the like.

Examples of an acyloxymethyl group represented by R² and R³ include an acetyloxymethyl group, a propionyloxymethyl group, a butyryloxymethyl group, an isobutyryloxymethyl group, a valeryloxymethyl group, an isovaleryloxymethyl group, a pivaloyloxymethyl group, and the like.

Examples of an acylthioethyl group represented by R² and R³ include an acetylthioethyl group, a propionylthioethyl group, a butyrylthioethyl group, an isobutyrylthioethyl group, a valerylthioethyl group, an isovalerylthioethyl group, a

pivaloylthioethyl group, and the like.

In an ethyl group substituted by one or more halogen atoms represented by R^2 and R^3 , the type of the halogen atom may be any of a fluorine, chlorine, bromine or iodine atom. Examples of an ethyl group substituted by one or more halogen atoms include a 1-fluoroethyl group, a 2-fluoroethyl group, a 1-chloroethyl group, a 2-chloroethyl group, a 2-bromoethyl group, a 2,2-difluoroethyl group, a 2,2-dichloroethyl group, a 2,2-dibromoethyl group, a 2,2,2-trifluoroethyl group, a 2,2,2-trichloroethyl group, a 2,2,2-tribromoethyl group, and the like. It is particularly preferred that the 2-position of an ethyl group is substituted, and the preferred halogen atom is a fluorine atom. At least one of R^2 and R^3 is preferably an ethyl group substituted by one or more halogen atoms, and particularly preferably 2,2,2-trifluoroethyl group.

Examples of a C_1 - C_4 alkyl group represented by R^4 include a methyl group, an ethyl group, an n-propyl group, an isopropyl group, an n-butyl group, an isobutyl group, a sec-butyl group, a tert-butyl group, and the like. Examples of a C_1 - C_4 hydroxyalkyl group represented by R^4 include a hydroxymethyl group, a 1-hydroxyethyl group, a 2-hydroxyethyl group, a 1-hydroxypropyl group, a 2-hydroxypropyl group, a 3-hydroxypropyl group, a 1-hydroxybutyl group, a 2-hydroxybutyl group, a 3-hydroxybutyl group, a 4-hydroxybutyl group, and the like. Examples of a C_1 - C_4 alkyl group substituted by one or more halogen atoms represented by R^4 include a group in which a halogen atom(s) such as a fluorine atom or a chlorine atom is/are bound to a methyl group, an ethyl group, an n-propyl group, an isopropyl group, an n-butyl group, an isobutyl group, a sec-butyl group, a tert-butyl group, and the like. Specific examples of such a group include a fluoromethyl group, a difluoromethyl group, a trifluoromethyl group, a fluoroethyl group, a chloroethyl group, a fluoropropyl group, a chloropropyl group, a fluorobutyl group, a chlorobutyl group, and the like.

The first condition for the preferred compound of the present invention is that each of R^2 and R^3 is independently a hydrogen atom, a C_1 - C_{22} alkyl group or an ethyl

group substituted by one or more halogen atoms. The second condition for the preferred compound of the present invention is that R^2 and R^3 is independently a hydrogen atom, a C_1 - C_{22} alkyl group or a 2,2,2-trifluoroethyl group, and R^4 is a hydrogen atom or a methyl group.

Examples of specific preferred compounds satisfying such conditions include the following compounds:

2-amino-6-(4-methoxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine bis(2,2,2-trifluoroethyl) ester;

2-amino-6-(4-methoxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine (2,2,2-trifluoroethyl) ester;

2-amino-6-(4-methoxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine;

2-amino-6-(4-hydroxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine bis(2,2,2-trifluoroethyl) ester;

2-amino-6-(3-hydroxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine bis(2,2,2-trifluoroethyl) ester;

2-amino-6-(2-hydroxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine bis(2,2,2-trifluoroethyl) ester;

2-amino-6-(4-hydroxyphenylthio)-9-[2-(phosphonomethoxy)propyl]purine bis(2,2,2-trifluoroethyl) ester;

2-amino-6-(3-hydroxyphenylthio)-9-[2-(phosphonomethoxy)propyl]purine bis(2,2,2-trifluoroethyl) ester;

2-amino-6-(2-hydroxyphenylthio)-9-[2-(phosphonomethoxy)propyl]purine bis(2,2,2-trifluoroethyl) ester;

2-amino-6-(4-hydroxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine methyl(2,2,2-trifluoroethyl) ester;

2-amino-6-(3-hydroxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine methyl(2,2,2-trifluoroethyl) ester;

2-amino-6-(2-hydroxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine methyl(2,2,2-trifluoroethyl) ester;

2-amino-6-(4-hydroxyphenylthio)-9-[2-(phosphonomethoxy)propyl]purine methyl(2,2,2-trifluoroethyl) ester;

2-amino-6-(3-hydroxyphenylthio)-9-[2-(phosphonomethoxy)propyl]purine methyl(2,2,2-trifluoroethyl) ester;

2-amino-6-(2-hydroxyphenylthio)-9-[2-(phosphonomethoxy)propyl]purine methyl(2,2,2-trifluoroethyl) ester;

2-amino-6-(4-hydroxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine (2,2,2-trifluoroethyl) ester;

2-amino-6-(3-hydroxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine (2,2,2-trifluoroethyl) ester;

2-amino-6-(2-hydroxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine (2,2,2-trifluoroethyl) ester;

2-amino-6-(4-hydroxyphenylthio)-9-[2-(phosphonomethoxy)propyl]purine (2,2,2-trifluoroethyl) ester;

2-amino-6-(3-hydroxyphenylthio)-9-[2-(phosphonomethoxy)propyl]purine (2,2,2-trifluoroethyl) ester;

2-amino-6-(2-hydroxyphenylthio)-9-[2-(phosphonomethoxy)propyl]purine (2,2,2-trifluoroethyl) ester;

2-amino-6-(4-hydroxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine;

2-amino-6-(3-hydroxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine;

2-amino-6-(2-hydroxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine;

2-amino-6-(4-hydroxyphenylthio)-9-[2-(phosphonomethoxy)propyl]purine;

2-amino-6-(3-hydroxyphenylthio)-9-[2-(phosphonomethoxy)propyl]purine; and

2-amino-6-(2-hydroxyphenylthio)-9-[2-(phosphonomethoxy)propyl]purine.

The third condition for the preferred compound of the present invention is that each of R^2 and R^3 is a 2,2,2-trifluoroethyl group and R^4 is a hydrogen atom or a methyl group. Examples of preferred compounds satisfying such conditions include the following compounds:

2-amino-6-(4-methoxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine bis(2,2,2-

trifluoroethyl) ester;
2-amino-6-(4-hydroxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine;
2-amino-6-(4-methoxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine;
2-amino-6-(4-hydroxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine
bis(2,2,2-trifluoroethyl) ester;
2-amino-6-(3-hydroxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine
bis(2,2,2-trifluoroethyl) ester;
2-amino-6-(2-hydroxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine
bis(2,2,2-trifluoroethyl) ester;
2-amino-6-(4-hydroxyphenylthio)-9-[2-(phosphonomethoxy)propyl]purine
bis(2,2,2-trifluoroethyl) ester;
2-amino-6-(3-hydroxyphenylthio)-9-[2-(phosphonomethoxy)propyl]purine
bis(2,2,2-trifluoroethyl) ester; and
2-amino-6-(2-hydroxyphenylthio)-9-[2-(phosphonomethoxy)propyl]purine
bis(2,2,2-trifluoroethyl) ester.

The fourth condition for the preferred compound of the present invention is that R^1 is a hydroxyl group, each of R^2 and R^3 is a 2,2,2-trifluoroethyl group, and R^4 is a hydrogen atom. Examples of preferred compounds satisfying such conditions include the following compounds:

2-amino-6-(4-hydroxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine
bis(2,2,2-trifluoroethyl) ester;
2-amino-6-(3-hydroxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine
bis(2,2,2-trifluoroethyl) ester; and
2-amino-6-(2-hydroxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine
bis(2,2,2-trifluoroethyl) ester.

The phosphonate nucleotide compound of the above formula (I) of the present invention may exist as a salt, and any salt formed by the above compound can be used as an active ingredient of the anti-viral agent of the present invention. Examples of such a salt include a pharmaceutically acceptable salt. Where an acidic group exists,

the acidic group is able to form metal salts such as a lithium salt, a sodium salt, a potassium salt, a magnesium salt and a calcium salt, and ammonium salts such as an ammonium salt, a methyl ammonium salt, a dimethyl ammonium salt, a trimethyl ammonium salt and dicyclohexyl ammonium salt. Where an amino group exists, the amino group is able to form mineral acid salts such as hydrochloride, hydrobromide, sulfate, nitrate, phosphate and metaphosphate, and organic acid salts such as methanesulfonate, benzenesulfonate, para-toluenesulfonate, acetate, propionate, tartrate, fumarate, maleate, malate, oxalate, succinate, citrate, benzoate, mandelate, cinnamate, lactate, besylate, valerate, stearate, oleate, lactobionate, ethylsuccinate, semisuccinate, butyrate, palmitate, carbamate, gluconate, laurate, salicylate, laokurate, tannate and butylsulfonate.

The phosphonate nucleotide compound of the above formula (I) and salt thereof may exist in the form of a hydrate or solvate. Any given hydrate or solvate which is formed by the phosphonate nucleotide compound of the above formula (I) or salt thereof, can be used as an active ingredient of the medicament of the present invention. Examples of a solvent capable of forming the solvate include methanol, ethanol, isopropanol, acetone, ethyl acetate, methylene chloride, diisopropyl ether, and the like.

Specific examples of the compounds of the formula (I) are shown in the following Table 1 (wherein Me- denotes a methyl group, Et- denotes an ethyl group, i-Pr- denotes an isopropyl group, and t-Bu- denotes a tert-butyl group).

Table 1

Table 1.

No.	R ¹	R ²	R ³	R ⁴	X
1	2-OH	CF ₃ CH ₂ -	CF ₃ CH ₂ -	H	CH
2	3-OH	CF ₃ CH ₂ -	CF ₃ CH ₂ -	H	CH
3	4-OH	CF ₃ CH ₂ -	CF ₃ CH ₂ -	H	CH
4	2-OH	CF ₃ CH ₂ -	CF ₃ CH ₂ -	H	N
5	3-OH	CF ₃ CH ₂ -	CF ₃ CH ₂ -	H	N
6	4-OH	CF ₃ CH ₂ -	CF ₃ CH ₂ -	H	N
7	2-OH	CF ₃ CH ₂ -	Me-	H	CH

8	3-OH	CF ₃ CH ₂ -	Me-	H	CH
9	4-OH	CF ₃ CH ₂ -	Me-	H	CH
10	2-OH	CF ₃ CH ₂ -	Me-	H	N
11	3-OH	CF ₃ CH ₂ -	Me-	H	N
12	4-OH	CF ₃ CH ₂ -	Me-	H	N
13	2-OH	CF ₃ CH ₂ -	Et-	H	CH
14	3-OH	CF ₃ CH ₂ -	Et-	H	CH
15	4-OH	CF ₃ CH ₂ -	Et-	H	CH
16	2-OH	CF ₃ CH ₂ -	H	H	CH
17	3-OH	CF ₃ CH ₂ -	H	H	CH
18	4-OH	CF ₃ CH ₂ -	H	H	CH
19	2-OH	CF ₃ CH ₂ -	H	H	N
20	3-OH	CF ₃ CH ₂ -	H	H	N
21	4-OH	CF ₃ CH ₂ -	H	H	N
22	2-OH	H	H	H	CH
23	3-OH	H	H	H	CH
24	4-OH	H	H	H	CH
25	2-OH	H	H	H	N
26	3-OH	H	H	H	N
27	4-OH	H	H	H	N
28	2-OH	CF ₃ CH ₂ -	CF ₃ CH ₂ -	Me-	CH
29	3-OH	CF ₃ CH ₂ -	CF ₃ CH ₂ -	Me-	CH
30	4-OH	CF ₃ CH ₂ -	CF ₃ CH ₂ -	Me-	CH
31	2-OH	CF ₃ CH ₂ -	CF ₃ CH ₂ -	Me-	N
32	3-OH	CF ₃ CH ₂ -	CF ₃ CH ₂ -	Me-	N
33	4-OH	CF ₃ CH ₂ -	CF ₃ CH ₂ -	Me-	N
34	2-OH	CF ₃ CH ₂ -	Me-	Me-	CH
35	3-OH	CF ₃ CH ₂ -	Me-	Me-	CH
36	4-OH	CF ₃ CH ₂ -	Me-	Me-	CH
37	2-OH	CF ₃ CH ₂ -	Me-	Me-	N
38	3-OH	CF ₃ CH ₂ -	Me-	Me-	N
39	4-OH	CF ₃ CH ₂ -	Me-	Me-	N
40	2-OH	CF ₃ CH ₂ -	Et-	Me-	CH
41	3-OH	CF ₃ CH ₂ -	Et-	Me-	CH
42	4-OH	CF ₃ CH ₂ -	Et-	Me-	CH
43	2-OH	CF ₃ CH ₂ -	Et-	Me-	N
44	3-OH	CF ₃ CH ₂ -	Et-	Me-	N
45	4-OH	CF ₃ CH ₂ -	Et-	Me-	N
46	2-OH	CF ₃ CH ₂ -	H	Me-	CH
47	3-OH	CF ₃ CH ₂ -	H	Me-	CH
48	4-OH	CF ₃ CH ₂ -	H	Me-	CH
49	2-OH	CF ₃ CH ₂ -	H	Me-	N
50	3-OH	CF ₃ CH ₂ -	H	Me-	N

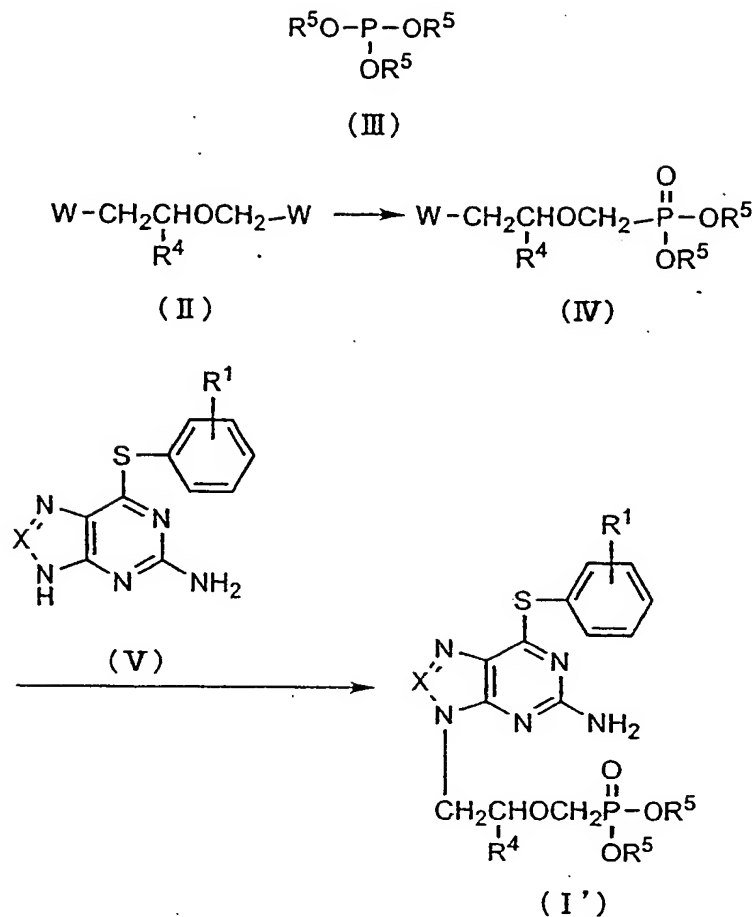
51	4-OH	CF ₃ CH ₂ -	H	Me-	N
52	2-OH	H	H	Me-	CH
53	3-OH	H	H	Me-	CH
54	4-OH	H	H	Me-	CH
55	2-OH	H	H	Me-	N
56	3-OH	H	H	Me-	N
57	4-OH	H	H	Me-	N
58	2-OH	-CH ₂ O-CO-t-Bu	-CH ₂ O-CO-t-Bu	H	CH
59	3-OH	-CH ₂ O-CO-t-Bu	-CH ₂ O-CO-t-Bu	H	CH
60	4-OH	-CH ₂ O-CO-t-Bu	-CH ₂ O-CO-t-Bu	H	CH
61	2-OH	-CH ₂ O-CO-t-Bu	-CH ₂ O-CO-t-Bu	Me-	CH
62	3-OH	-CH ₂ O-CO-t-Bu	-CH ₂ O-CO-t-Bu	Me-	CH
63	4-OH	-CH ₂ O-CO-t-Bu	-CH ₂ O-CO-t-Bu	Me-	CH
64	2-OH	-CH ₂ CH ₂ S-CO-i-Pr	-CH ₂ CH ₂ S-CO-i-Pr	H	CH
65	3-OH	-CH ₂ CH ₂ S-CO-i-Pr	-CH ₂ CH ₂ S-CO-i-Pr	H	CH
66	4-OH	-CH ₂ CH ₂ S-CO-i-Pr	-CH ₂ CH ₂ S-CO-i-Pr	H	CH
67	2-OH	-CH ₂ CH ₂ S-CO-i-Pr	-CH ₂ CH ₂ S-CO-i-Pr	Me-	CH
68	3-OH	-CH ₂ CH ₂ S-CO-i-Pr	-CH ₂ CH ₂ S-CO-i-Pr	Me-	CH
69	4-OH	-CH ₂ CH ₂ S-CO-i-Pr	-CH ₂ CH ₂ S-CO-i-Pr	Me-	CH
70	2-OCH ₃	CF ₃ CH ₂ -	CF ₃ CH ₂ -	H	CH
71	3-OCH ₃	CF ₃ CH ₂ -	CF ₃ CH ₂ -	H	CH
72	4-OCH ₃	CF ₃ CH ₂ -	CF ₃ CH ₂ -	H	CH
73	2-OCH ₃	CF ₃ CH ₂ -	CF ₃ CH ₂ -	H	N
74	3-OCH ₃	CF ₃ CH ₂ -	CF ₃ CH ₂ -	H	N
75	4-OCH ₃	CF ₃ CH ₂ -	CF ₃ CH ₂ -	H	N
76	2-OCH ₃	CF ₃ CH ₂ -	Me-	H	CH
77	3-OCH ₃	CF ₃ CH ₂ -	Me-	H	CH
78	4-OCH ₃	CF ₃ CH ₂ -	Me-	H	CH
79	2-OCH ₃	CF ₃ CH ₂ -	Me-	H	N
80	3-OCH ₃	CF ₃ CH ₂ -	Me-	H	N
81	4-OCH ₃	CF ₃ CH ₂ -	Me-	H	N
82	2-OCH ₃	CF ₃ CH ₂ -	Et-	H	CH
83	3-OCH ₃	CF ₃ CH ₂ -	Et-	H	CH
84	4-OCH ₃	CF ₃ CH ₂ -	Et-	H	CH
85	2-OCH ₃	CF ₃ CH ₂ -	H	H	CH
86	3-OCH ₃	CF ₃ CH ₂ -	H	H	CH
87	4-OCH ₃	CF ₃ CH ₂ -	H	H	CH
88	2-OCH ₃	CF ₃ CH ₂ -	H	H	N
89	3-OCH ₃	CF ₃ CH ₂ -	H	H	N
90	4-OCH ₃	CF ₃ CH ₂ -	H	H	N
91	2-OCH ₃	H	H	H	CH
92	3-OCH ₃	H	H	H	CH
93	4-OCH ₃	H	H	H	CH

94	2-OCH ₃	H	H	H	N
95	3-OCH ₃	H	H	H	N
96	4-OCH ₃	H	H	H	N
97	2-OCH ₃	CF ₃ CH ₂ -	CF ₃ CH ₂ -	Me-	CH
98	3-OCH ₃	CF ₃ CH ₂ -	CF ₃ CH ₂ -	Me-	CH
99	4-OCH ₃	CF ₃ CH ₂ -	CF ₃ CH ₂ -	Me-	CH
100	2-OCH ₃	CF ₃ CH ₂ -	CF ₃ CH ₂ -	Me-	N
101	3-OCH ₃	CF ₃ CH ₂ -	CF ₃ CH ₂ -	Me-	N
102	4-OCH ₃	CF ₃ CH ₂ -	CF ₃ CH ₂ -	Me-	N
103	2-OCH ₃	CF ₃ CH ₂ -	Me-	Me-	CH
104	3-OCH ₃	CF ₃ CH ₂ -	Me-	Me-	CH
105	4-OCH ₃	CF ₃ CH ₂ -	Me-	Me-	CH
106	2-OCH ₃	CF ₃ CH ₂ -	Me-	Me-	N
107	3-OCH ₃	CF ₃ CH ₂ -	Me-	Me-	N
108	4-OCH ₃	CF ₃ CH ₂ -	Me-	Me-	N
109	2-OCH ₃	CF ₃ CH ₂ -	Et-	Me-	CH
110	3-OCH ₃	CF ₃ CH ₂ -	Et-	Me-	CH
111	4-OCH ₃	CF ₃ CH ₂ -	Et-	Me-	CH
112	2-OCH ₃	CF ₃ CH ₂ -	Et-	Me-	N
113	3-OCH ₃	CF ₃ CH ₂ -	Et-	Me-	N
114	4-OCH ₃	CF ₃ CH ₂ -	Et-	Me-	N
115	2-OCH ₃	CF ₃ CH ₂ -	H	Me-	CH
116	3-OCH ₃	CF ₃ CH ₂ -	H	Me-	CH
117	4-OCH ₃	CF ₃ CH ₂ -	H	Me-	CH
118	2-OCH ₃	CF ₃ CH ₂ -	H	Me-	N
119	3-OCH ₃	CF ₃ CH ₂ -	H	Me-	N
120	4-OCH ₃	CF ₃ CH ₂ -	H	Me-	N
121	2-OCH ₃	H	H	Me-	CH
122	3-OCH ₃	H	H	Me-	CH
123	4-OCH ₃	H	H	Me-	CH
124	2-OCH ₃	H	H	Me-	N
125	3-OCH ₃	H	H	Me-	N
126	4-OCH ₃	H	H	Me-	N
127	2-OCH ₃	-CH ₂ O-CO-t-Bu	-CH ₂ O-CO-t-Bu	H	CH
128	3-OCH ₃	-CH ₂ O-CO-t-Bu	-CH ₂ O-CO-t-Bu	H	CH
129	4-OCH ₃	-CH ₂ O-CO-t-Bu	-CH ₂ O-CO-t-Bu	H	CH
130	2-OCH ₃	-CH ₂ O-CO-t-Bu	-CH ₂ O-CO-t-Bu	Me-	CH
131	3-OCH ₃	-CH ₂ O-CO-t-Bu	-CH ₂ O-CO-t-Bu	Me-	CH
132	4-OCH ₃	-CH ₂ O-CO-t-Bu	-CH ₂ O-CO-t-Bu	Me-	CH
133	2-OCH ₃	-CH ₂ CH ₂ S-CO-i-Pr	-CH ₂ CH ₂ S-CO-i-Pr	H	CH
134	3-OCH ₃	-CH ₂ CH ₂ S-CO-i-Pr	-CH ₂ CH ₂ S-CO-i-Pr	H	CH
135	4-OCH ₃	-CH ₂ CH ₂ S-CO-i-Pr	-CH ₂ CH ₂ S-CO-i-Pr	H	CH
136	2-OCH ₃	-CH ₂ CH ₂ S-CO-i-Pr	-CH ₂ CH ₂ S-CO-i-Pr	Me-	CH

137	3-OCH ₃	-CH ₂ CH ₂ S-CO-i-Pr	-CH ₂ CH ₂ S-CO-i-Pr	Me- CH
138	4-OCH ₃	-CH ₂ CH ₂ S-CO-i-Pr	-CH ₂ CH ₂ S-CO-i-Pr	Me- CH

As a production method of the compound of the formula (I) in the case where in the formula (I), each of R² and R³ is a C₁-C₂₂ alkyl group, or an ethyl group substituted by one or more halogen atoms, the compound can be synthesized, for example, according to the following reaction route (1) or (2). In the following scheme, R¹, R⁴ and X are the same as defined above, R⁵ represents a C₁-C₂₂ alkyl group, or an ethyl group substituted by one or more halogen atoms, and W represents a leaving group such as a halogen atom, a para-toluenesulfonyloxy group, a methanesulfonyloxy group or a trifluoromethanesulfonyloxy group.

<Reaction Route (1)>



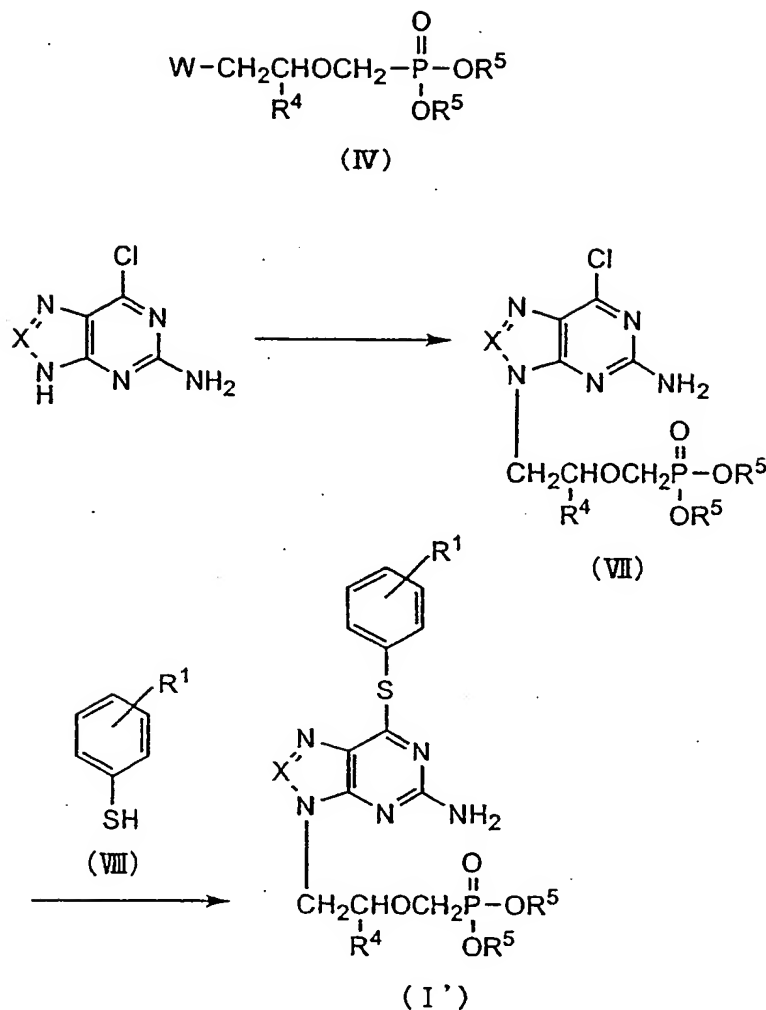
First, the compound of the above formula (II) is reacted with the compound of the above formula (III) at a temperature of 10°C to 250°C, preferably 130°C to 200°C, for 0.1 to 100 hours, preferably for 3 to 24 hours. The compound of the above formula (IV) obtained by the above reaction can be separated and purified by ordinary separation and purification means such as distillation-, adsorption- or partition-chromatographies, as necessary. The compound of the above formula (IV) may be separated and purified as stated above, or it may directly be used for the following reaction without purification. The compound of the above formula (IV) is

reacted with the compound of the above formula (V) in the presence of a base such as sodium carbonate, potassium carbonate, cesium carbonate, sodium hydrate, potassium hydrate, triethylamine and diazabicycloundecen in a suitable solvent such as acetonitrile, tetrahydrofuran, dimethylsulfoxide, dimethylformamide or methylpyrrolidone at a temperature of 10°C to 200°C, preferably 50°C to 150°C, for 0.1 to 100 hours, preferably for 1 to 10 hours, to obtain the compound of the above formula (I').

The source of the compounds of the above formulas (II), (III) and (V) which are raw materials for reaction route (1) is not particularly limited. For example, a compound commercially available as a reagent may be used, or a compound may be synthesized by a known method, as appropriate. By way of example, the compound of the above formula (V) can be synthesized by heating the compound of the formula (VI) and the compound of the formula (VIII) which are described later, in a suitable solvent such as acetonitrile or dimethylsulfoxide at a range of 50°C to 100°C.

The compound of the above formula (I') can also be produced by the following method. In the following scheme, R¹, R⁴, R⁵, X and W are the same as defined above.

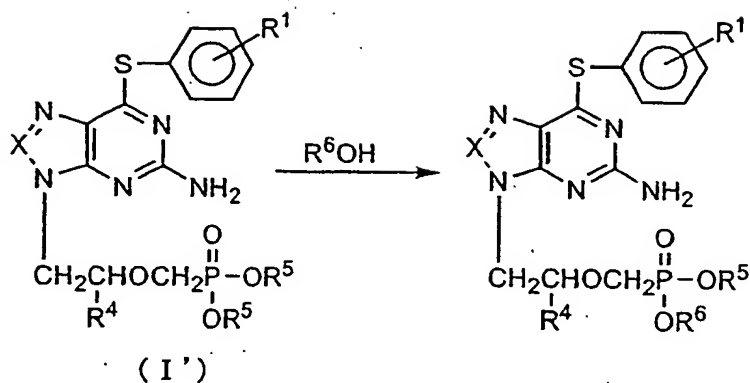
<Reaction Route (2)>



The compound of the above formula (IV) obtained by reaction route (1) is reacted with the compound of the above formula (VI) in the presence of a base such as sodium carbonate, potassium carbonate, cesium carbonate, sodium hydrate, potassium hydrate, triethylamine and diazabicycloundecen in a suitable solvent such as acetonitrile, tetrahydrofuran, dimethylsulfoxide, dimethylformamide or methylpyrrolidone at a temperature of 10°C to 200°C, preferably 50°C to 150°C for 0.1 to 100 hours,

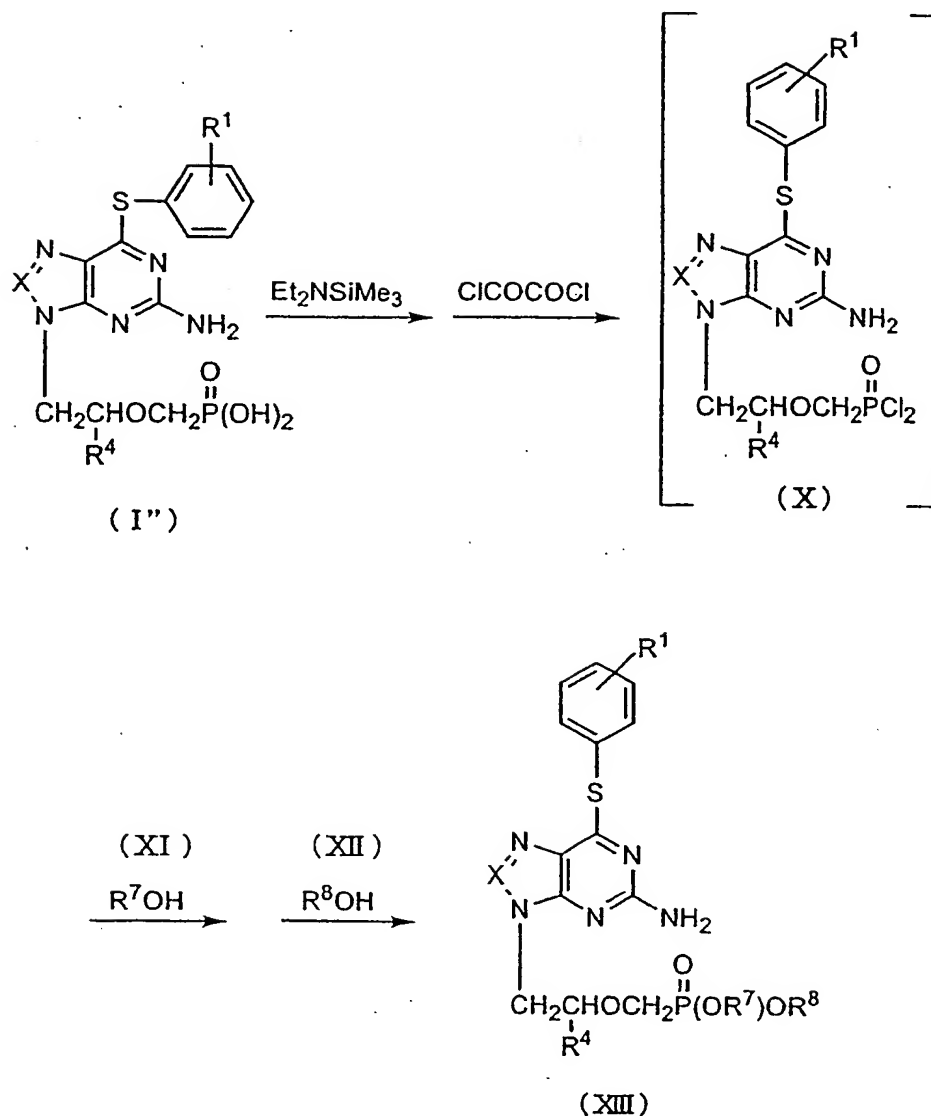
preferably for 0.5 to 10 hours, to obtain the compound of the above formula (VII). Thereafter, the compound of the above formula (VII) is reacted with a mercaptan represented by the above formula (VIII) or a salt thereof such as a sodium salt, a potassium salt, a lithium salt or a triethylamine salt, in a suitable solvent such as acetonitrile, tetrahydrofuran, dimethylsulfoxide, dimethylformamide or methylpyrrolidone optionally in the presence of a suitable tertiary amine at a temperature of 10°C to 200°C, preferably 70°C to 120°C for 0.1 to 100 hours, preferably for 0.5 to 12 hours, to obtain the compound of the above formula (I'). The compound of the formula (I') corresponds to a compound of the formula (I) wherein each of R² and R³ is a C₁-C₂₂ alkyl group, or an ethyl group substituted by one or more halogen atoms. The source of the compound of the above formula (VI) which is a raw material of reaction route (2), is not particularly limited. For example, a compound commercially available as a reagent may be used, or the compound may be synthesized by a known method as appropriate.

By further altering a phosphate ester portion of the compound of the above formula (I'), there can be obtained the compound of the formula (I) wherein R⁵ of the compound of the formula (I') is converted into another substituent. For example, a compound of the formula (I) wherein both R² and R³ are hydrogen atoms can be obtained by hydrolysis of the compound of the above formula (I'). Moreover, a compound of the formula (I) wherein R³ is a hydrogen atom, a C₁-C₂₂ alkyl group, an acylthioethyl group, or an ethyl group substituted by one or more halogen atoms, and R² is a C₁-C₂₂ alkyl group, or an ethyl group substituted by one or more halogen atoms, can be obtained by reaction of the compound of the above formula (I') with the compound of the formula (IX): R⁶OH wherein R⁶ is a hydrogen atom, a C₁-C₂₂ alkyl group, an acylthioethyl group, or an ethyl group substituted by one or more halogen atoms, in no solvent or in a suitable solvent including a chloric solvent such as dichloromethane; pyridine; acetonitrile; tetrahydrofuran; dimethylsulfoxide; dimethylformamide and methylpyrrolidone, optionally in the presence of acid or alkali, at a temperature of 10°C to 100°C, preferably 20°C to 30°C, for 0.1 to 100 hours, preferably 5 to 12 hours.



In the above scheme, R^1 , R^4 , R^5 , R^6 and X are the same as defined above.

A compound of the formula (I) wherein each of R^2 and R^3 is independently a hydrogen atom, a C_1 - C_{22} alkyl group, an acylthioethyl group, or an ethyl group substituted by one or more halogen atoms, can also be obtained by the following method. In the following scheme, R^1 , R^4 and X are the same as defined above, and each of R^7 and R^8 is independently a hydrogen atom, a C_1 - C_{22} alkyl group, an acylthioethyl group, or an ethyl group substituted by one or more halogen atoms, with the exception that both R^7 and R^8 can not represent hydrogen atoms at the same time.



First, the compound of the above formula (I'') is reacted with trimethylsilyldiethylamine in a suitable solvent such as dichloromethane, dichloroethane and chloroform around room temperature for about 1 hour. More than two moles of trimethylsilyldiethylamine are used per mole of the compound of the above formula (I''). After the reaction solution is concentrated to dryness, the residue is dissolved into a suitable solvent, for example, a chloric solvent such as dichloromethane, and then oxalyl chloride is added in an amount of 2 or more moles per mole of the compound of

the above formula (I''), followed by reaction on ice for about 1 hour, and then around room temperature for about 1 hour in the presence of a catalytic amount of dimethylformamide.

The compound of the above formula (X) obtained by removal of the solvent, usually without being purified, is reacted with the compound of the formula (XI) and/or the compound of the formula (XII) in a suitable solvent, for example, a chloric solvent such as dichloromethane, pyridine, acetonitrile, tetrahydrofuran, dimethylsulfoxide, dimethylformamide or methylpyrrolidone, at a temperature of 10°C to 100°C, preferably 20°C to 30°C for 0.1 to 100 hours, preferably 5 to 12 hours. The obtained compound of the formula (XIII) corresponds to a compound of the formula (I) wherein each of R^2 and R^3 is independently a hydrogen atom, a C_1 - C_{22} alkyl group, an acylthioethyl group, or an ethyl group substituted by one or more halogen atoms with the exception that both R^2 and R^3 can not represent hydrogen atoms at the same time. As stated above, the compound of the above formula (I'') which is a raw material of the above reaction, can be obtained by hydrolysis of the compound of the formula (I'), or it can be more efficiently obtained by reaction of a compound of the formula (I') wherein R^5 is a C_1 - C_{22} alkyl group with triethyliodosilane, trimethylbromosilane and the like.

A compound of the formula (I) wherein each of R^2 and R^3 is an acyloxymethyl group, or wherein either one of R^2 and R^3 is an acyloxymethyl group and the other is hydrogen, can be obtained by reaction of the compound of the above formula (I'') with an acyloxymethyl halide represented by the following formula (XIV): R^9Y wherein R^9 is an acyloxymethyl group and Y is a chlorine, bromine or iodine atom, in the presence of a base such as sodium carbonate, potassium carbonate, cesium carbonate, sodium hydride, potassium hydride, triethylamine, pyridine, diazabicycloundecen and N,N'-dichlorohexyl-4-morpholinecarboxamidine in a suitable solvent such as acetonitrile, tetrahydrofuran, dimethylsulfoxide, dimethylformamide or methylpyrrolidone at a temperature of 0°C to 200°C, preferably 10°C to 100°C for 1 to 300 hours, preferably for 10 to 200 hours. In the case of a compound wherein each of R^2 and R^3 is an acyloxymethyl group, the compound of the formula (XIV) may be

reacted with the compound of the formula (I'') in an amount of 2 moles per mole of the compound of the formula (I'), while in the case of a compound wherein either one is an acyloxymethyl group, an equivalent mole reaction may be applied.

A compound wherein either one of R^2 and R^3 is an acyloxymethyl group, and the other is a C_1 - C_{22} alkyl group, an acylthioethyl group, or an ethyl group substituted by one or more halogen atoms, can be produced by preparing a compound wherein either one of R^2 and R^3 is a C_1 - C_{22} alkyl group, an acylthioethyl group or an ethyl group substituted by one or more halogen atoms, and the other is a hydrogen atom, and then reacting the compound of the above formula (XIV) to this compound by the above method.

The salt of the compound of the formula (I) can be synthesized, for example, by the following method. The compound of the formula (I') is reacted with a corresponding acid with stirring at a temperature of -10°C to 100°C , preferably 10°C to 50°C for 0.1 to 20 hours, preferably for 0.3 to 1 hour in a suitable solvent such as ethyl acetate, isopropanol, acetonitrile, tetrahydrofuran, dimethylsulfoxide, dimethylformamide or methylpyrrolidone.

Among compounds represented by the formula (I), compounds wherein R^1 is a hydroxyl group are disclosed in Japanese Patent Application No. 2000-54675 (published as WO01/64693) and PCT/JP01/01412, and compounds wherein R^1 is a C_1 - C_6 alkoxy group are disclosed in Japanese Patent Application Laid-Open (Kokai) No. 9-255695 (Patent No. 3148139). The disclosures of these applications are incorporated herein as a part of the disclosed of the present application.

The above production method is provided as an example of a method for producing the compound of general formula (I), and so the method for producing the compound used in the present invention is not limited thereto. The compound of the above formula (I) produced by the above method or a salt thereof can be separated and purified by ordinary nucleotide separation and purification means, e.g., by selecting and applying means such as recrystallization-, adsorption-, ion exchange- and partition-chromatographies, as appropriate.

A target virus to which the anti-viral agent of the present invention is applicable is not particularly limited, and specific examples of the target virus include an RNA virus such as human immunodeficiency virus, influenza virus and hepatitis C virus; and a DNA virus such as herpes simplex virus type 1 and type 2, cytomegalovirus, varicella-zoster virus and hepatitis B virus, with hepatitis B virus being more preferable.

Where the anti-viral agent of the present invention is used as a medicament, the compound of the formula (I) may be used singly, but it is preferred that, using a pharmacologically acceptable pharmaceutical additive, a pharmaceutical composition comprising the above compound as an active ingredient is produced and administered. The composition of the pharmaceutical composition is determined by the solubility of the compound, chemical properties, administration route, dosage regimen and the like. For example, the compound can be orally administered in an dosage form of a granule, a parvule, a powder, a tablet, a hard syrup, a soft capsule, a troche, a syrup, an emulsion, a soft gelatine capsule, a gel, a paste, a suspension, a liposome and the like, or the compound can be administered intravenously, intramuscularly or subcutaneously in the form of an injection. In addition, the compound may be formulated into powders for injection, and a solution may be prepared before use.

As a pharmacologically acceptable pharmaceutical additive, an organic or inorganic, solid or liquid carrier, which is suitable for oral, enteral, parenteral or local administration, can be used. Examples of a solid carrier used for the production of a solid formulation include lactose, sucrose, starch, talc, cellulose, dextrin, kaoline, calcium carbonate, agar, pectin, stearic acid, magnesium stearate, lecithin, and sodium chloride. Examples of a liquid carrier used for the production of a liquid formulation for oral administration include glycerine, peanut oil, polyvinylpyrrolidone, olive oil, ethanol, benzyl alcohol, propylene glycol, physiological saline, and water. The above pharmaceutical composition can also comprise, in addition to the above carriers, an adjuvant such as a wetting agent, a suspension aid, a sweetener, a flavor, a coloring agent and a preservative. Further, a liquid agent may be contained in a capsule of a substance which can be absorbed, such as gelatin. Examples of a solvent or a

suspending agent, which is used for the production of a formulation for parenteral administration such as an injection, include water, propylene glycol, polyethylene glycol, benzyl alcohol, ethyl oleate, and lecithin.

Considering the properties of known compounds, it can easily be assumed that the compound of the formula (I), especially the ester derivative of the above formula (I') has a high oral absorbency, and therefore oral administration is a preferred administration route for the anti-viral agent of the present invention. The preparation of each of the above formulation can be carried out according to standard techniques. Where the anti-viral agent of the present invention is used for oral administration, the clinical dose is generally 0.1 to 500mg of the compound per kg adult per day, and preferably 0.1 to 50mg of the compound per kg adult per day. The dose may be changed as appropriate, depending on age, disease condition, symptom, the presence or absence of concurrent administration and the like. The above dose may be applied once a day or divided over two to several administrations per day at regular intervals, or may also be applied intermittently every several days. Where the compound of the formula (I) is used as an injection, the applied dose is 0.01 to 50mg of the compound per kg adult per day, preferably 0.1 to 5mg per kg.

[EXAMPLES]

The present invention is further described in the following examples. The present invention is not limited to the Examples. The compound numbers in the Examples correspond to those in Table 1.

Synthesis Example 1: Production of 2-amino-6-(4-hydroxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine bis(2,2,2-trifluoroethyl) ester (Compound No. 3)

87g of 2-chloroethylchloromethylether (670mmol) was reacted with 200g of Tris(2,2,2-trifluoroethyl)phosphite (610mmol) at 160°C for 7 hours to obtain 2-[bis(2,2,2-trifluoroethyl)phosphonylmethoxy]ethylchloride quantitatively.

206g of 2-(phosphonomethoxy)ethylchloride bis(2,2,2-trifluoroethyl) ester was dissolved in 2,000ml of methylethylketone, 270g of sodium iodide was added thereto, and the mixture was refluxed for 8 hours. After reaction, the mixture was cooled to room temperature followed by concentration to dryness. The residue was dissolved in chloroform/hexane and then was adsorbed to a silica gel column, followed by elution with chloroform/hexane to obtain 2-(phosphonomethoxy)ethyliodo bis(2,2,2-trifluoroethyl) ester quantitatively.

15.0g (88mmol) of 2-amino-6-chloropurine was suspended in 360ml of dimethylformamide, and the suspension was reacted with 13.9ml (93mmol) of 1,8-diazabicyclo[5.4.0]undec-7-ene at 80°C for 1 hour. 23.8ml of 2-(phosphonomethoxy)ethyliodo bis(2,2,2-trifluoroethyl) ester was added to the above reaction solution, and the mixture was reacted at 100°C for 5 hours. After reaction, the mixture was cooled to room temperature, followed by concentration to dryness. The residue was dissolved in chloroform, and was then adsorbed to a silica gel column followed by elution with 5%-methanol-chloroform to obtain 23.3g (yield 56%) of 2-amino-6-chloro-9-[2-(phosphonomethoxy)ethyl]purine bis(2,2,2-trifluoroethyl) ester.

To 10ml of dimethylformamide solution containing 2g of 2-amino-6-chloro-9-[2-(phosphonomethoxy)ethyl]purine bis(2,2,2-trifluoroethyl) ester, 0.8ml of pyridine and 0.64g of 4-hydroxythiophenol were added, and the mixture was stirred at 100°C for 2 hours. The reaction mixture was cooled to room temperature followed by concentration to dryness.

The residue was dissolved in chloroform and was then adsorbed to a silica gel column followed by elution with 5% to 20% methanol-chloroform to obtain 1.3g (yield 55%) of 2-amino-6-(4-hydroxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine bis(2,2,2-trifluoroethyl) ester.

¹H-NMR (DMSO-d₆, δ): 3.85-3.88 (m, 2H), 4.14 (d, J=8.1Hz, 2H), 4.19-4.22 (m, 2H), 4.62-4.71 (m, 4H), 6.27 (s, 2H), 6.84 (d, J=8.7Hz, 2H) 7.7 (d, J=8.7Hz, 2H), 7.89 (s, 1H), 9.85 (s, 1H)

Synthesis Example 2: Production of 2-amino-6-(4-hydroxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine methyl(2,2,2-trifluoroethyl) ester (Compound No. 9)

100mg of 2-amino-6-(4-hydroxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine bis(2,2,2-trifluoroethyl ester (Compound No. 3) was dissolved in a 0.35N ammonia methanol solution, and the mixture was left at room temperature for 40 minutes, followed by removal of the solvent by distillation to obtain the compound of interest.

¹H-NMR (DMSO-d₆, δ): 3.66 (d, J=4.5Hz, 3H), 3.83-3.87 (m, 2H), 4.00 (d, J=8.1Hz, 2H), 4.18-4.22 (m, 2H), 4.52-4.60 (m, 2H), 6.23 (s, 2H), 6.83 (d, J=8.4Hz, 2H), 7.37 (d, J=8.4Hz, 2H), 7.89 (s, 1H), 9.81 (s, 1H)

Synthesis Example 3: Production of 2-amino-6-(4-hydroxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine 2,2,2-trifluoroethyl ester (Compound No. 18)

60mg of 2-amino-6-(4-hydroxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purinebis2,2,2-trifluoroethyl ester (Compound No. 3) was dissolved in a 1N ammonia solution, and the mixture was left at room temperature for 3 hours, followed by removal of the solvent by distillation to obtain the compound of interest.

¹H-NMR (DMSO-d₆, δ): 3.51-3.54 (m, 2H), 3.74-3.77 (m, 2H), 4.03-4.12 (m, 2H), 4.14-4.16 (m, 2H), 6.20 (s, 2H), 6.82 (d, J=8.4Hz, 2H), 7.12 (b, 3H), 7.36 (d, J=8.4Hz, 2H), 8.00 (s, 1H), 9.81 (s, 1H)

Synthesis Example 4. Production of 2-amino-6-(4-hydroxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine (Compound No. 24)

2-amino-6-chloro-9-[2-(phosphonomethoxy)ethyl]purine bisisopropyl ester was obtained by the same process as in Example 1, with the only exception being that triisopropylphosphate was used for substitution of Tris(2,2,2-trifluoroethyl)phosphate.

4.1ml of bromotrimethylsilane was added to 37ml of acetonitrile solution containing 3.7g of 2-amino-9-[2-(phosphonomethoxy)ethyl]-6-chloropurine

bisisopropyl ester, and the mixture was stirred at 25°C for 16 hours. Then, the solvent was removed by vacuum removal and the residue was crystallized from 45ml acetone-15ml water to obtain 2.4g of 2-amino-6-chloro-9-[2-(phosphonomethoxy)ethyl]purine. 304mg of 4-hydroxythiophenol and 0.32ml of pyridine were added to 5ml of DMF solution containing 308mg of the obtained compound, and the mixture was heated at 100°C for 4 hours. After removal of the solvent, the compound of interest was isolated by high performance liquid chromatography.

¹H-NMR (DMSO-d₆, δ): 3.57-3.60 (m, 2H), 3.81-3.84 (m, 2H), 6.83 (d, J=8.7Hz, 2H), 7.38 (d, J=8.7Hz, 2H), 7.96 (s, 1H)

Test Example

(1) Preparation of RNA

The schematic diagram of plasmid constructs used in this test is shown in Figure 1. Each of the plasmid constructs has a T7, T3 or Sp6 promoter region upstream of a region encoding an RNA of interest. After digestion of each plasmid construct with restriction enzymes, using RiboMAXTM Large Scale RNA Production Systems (Promega) according to a manufacturer-recommended method, an RNA of interest was obtained. In a construct used in the conventional method, both a region encoding POL and a ϵ motif exist in the same RNA. In a construct used in the improved method, an RNA encoding POL and another RNA having a ϵ motif were synthesized separately. Free nucleotides were removed from the obtained RNA products by MicroSpin S-300HR column (Amersham), and the concentration of RNA was adjusted with nuclease-free water.

(2) Pharmaceutical evaluation test by the conventional method

The method of Staschke and Colacino (1994) was used with some modifications. Using an RNA obtained from the construct as shown in Figure 1A and Rabbit Reticulocyte Lysate System, nuclease treated (Promega), an in vitro translation

reaction was carried out to obtain a POL- ϵ RNA complex of interest. 50mM Tris-hydrochloride buffer (pH7.5), 15mM NaCl and 10mM MgCl₂ at final concentration were added to 2.5 μ l of POL- ϵ RNA complex, and dNTP and a test medicament were further added thereto to prepare the mixture of a final volume of 5 μ l, followed by incubation at 30°C for 30 minutes. In each test, either [α 32P] labeled dATP or dGTP with a specific activity of 400 to 1,000Ci/mmol was prepared and used. After incubation of the reaction mixture, the mixture was subjected to SDS polyacrylamide gel electrophoresis according to standard techniques, and the gel was dried, followed by exposure to an Imaging Plate (Fuji Film). The exposed Imaging Plate was read by BAS-5000 (Fuji Film), the labeled POL was detected, and the radioactivity was determined by deduction of the background value of each lane. The pharmaceutical competitive action of the labeled compound was analyzed using kinetics with a dNTP at final concentration of 2 to 0.1 μ M. The test results obtained by the conventional method are shown in Figure 2. At present, the oral prodrug of phosphonomethoxyethyl adenine (PMEA; general name: adefovir) is undergoing a clinical test for an anti-HBV agent; and the diphosphorylated form of PMEA (PMEApp) competes with dATP and proved to be an active metabolite of PMEA. In this experiment system, it was confirmed that PMEApp, diphosphorylated form of PMEA, competed with dATP. However, compound A (2-amino-6-(4-hydroxyphenylthio) 9-[2-(phosphonomethoxy)ethyl]purine) (described in Japanese Patent Application Laid-Open (Kokai) No. 9-255695 (Japanese Patent No. 3148139)) showed no inhibitory activity in this experiment system.

(3) Establishment of an improved method

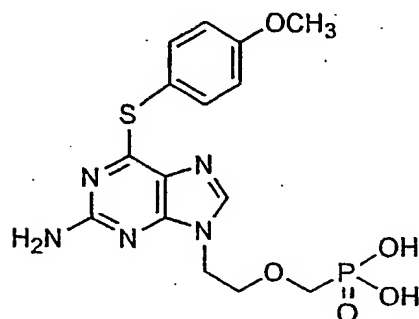
To find a compound which shows a pharmaceutical effect with a novel action mechanism such as a POL- ϵ RNA binding inhibitory agent or an inhibition of the first deoxynucleotide covalently attached to POL, which could not be found by the conventional method, the conventional experiment series was improved. Using an RNA obtained from the construct shown in Figure 1B and Rabbit Reticulocyte Lysate

System, nuclease treated (Promega), an in vitro translation reaction was carried out to obtain a POL protein alone, and then cycloheximide was added thereto at final concentration of 1mM to terminate the translation reaction. Similarly, a sample wherein translation is not terminated was prepared. Thereafter, 1/10 volume of ϵ RNA obtained from the construct shown in Figure 1C was added to the solution at final concentration of 500 to 5.12nM and the mixture was further incubated at 30°C for 60 minutes. To 2.5 μ l of the reaction mixture obtained as above, 50mM Tris-hydrochloride buffer (pH7.5), 15mM NaCl and 10mM $MgCl_2$ at final concentration were added, and [α 32P]-dGTP (600Ci/mmol) was further added thereto at final concentration of 1.6 μ M to prepare the mixture of a final volume of 5 μ l, followed by incubation at 30°C for 30 minutes. After incubation of the reaction solution, the solution was subjected to 0.1% SDS 7.5% polyacrylamide gel electrophoresis according to standard techniques, and the gel was dried, followed by exposure on an Imaging Plate (Fuji Film). The exposed Imaging Plate was read by BAS-5000 (Fuji Film), the labeled POL was detected, and the radioactive level was determined by deduction of the background value of each lane. The results are shown in Figure 3. The results of a double reciprocal plot (Lineweaver & Burk plot) of Figure 3B show that there is a clear correlation between POL and ϵ RNA concentration. It has become possible that a compound having a novel mechanism such as POL- ϵ RNA binding inhibition is evaluated. Furthermore, since the signal intensity differed according to whether or not cycloheximide was added, it was considered that where cycloheximide was not added, the translation reaction still continued at the point of ϵ RNA binding reaction. Therefore, it was considered that termination of the translation reaction by addition of cycloheximide is required before addition of a medicament and a ϵ RNA to prevent a pseudo-positive state caused by a non-specific translation inhibitor.

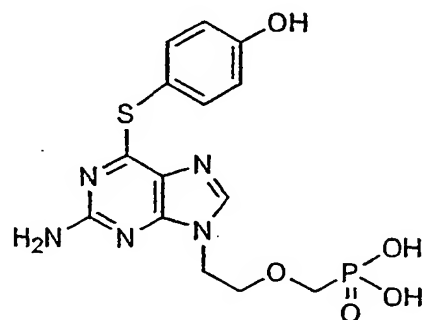
(4) Pharmaceutical evaluation test by the improved method

A test by the improved method was carried out by preparing POL according to the above-described method, adding cycloheximide thereto at final concentration of

1mM, and further adding a test medicament, followed by incubation at 30°C for 15 to 30 minutes. The test medicament used in the present invention were compound A (2-amino-6-(4-methoxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine) (described in Japanese Patent Application Laid-Open (Kokai) No. 9-255695 (Japanese Patent No. 3148139)) and compound B (2-amino-6-(4-hydroxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine (compound No. 24)). The structure of each of compounds A and B is shown below.



(Compound A)



(Compound B)

Thereafter, a ϵ RNA was added thereto and the mixture was further incubated at 30°C for 60 minutes. 50mM Tris-hydrochloride buffer (pH7.5), 15mM NaCl and 10mM $MgCl_2$ at final concentration were added to 2.5 μ l of the obtained reaction solution, and dNTP was further added thereto to obtain the mixture of a final volume of 5 μ l, followed by incubation at 30°C for 30 minutes. In each test, either [α ^{32}P] labeled dATP or dGTP with a specific activity of 400 to 1,000Ci/mmol was prepared and used. After incubation of the reaction solution, the solution was subjected to 0.1% SDS 7.5% polyacrylamide gel electrophoresis according to standard techniques, and the gel was dried, followed by exposure on an Imaging Plate (Fuji Film). The exposed Imaging Plate was read by BAS-5000 (Fuji Film), the labeled POL was detected, and the radioactive level was determined by deduction of the background

value of each lane. Figure 4 shows the results of kinetics analysis of ϵ RNA and Figure 5 shows the results of each binding reaction to ϵ RNA, in which POL itself was diluted with rabbit reticulocyte lysate to 1, 1/2, 1/3, 1/4 and 1/5, followed by addition of a medicament. Compound A showed no inhibitory activity in the conventional method (Figure 2), but showed the activity in the improved method. The test results obtained by each of the conventional and improved methods are shown below (Table 2).

Table 2.

Summary of analyses by conventional and improved methods

	Analysis result by the conventional method		Analysis result by the improved method	
	dGTP	dATP	ϵ RNA competition	POL competition
PMEA	negative	negative	negative	negative
PMEApp	negative	positive	no tests	no test
Compound A	negative	negative	positive	no tests
Compound B	negative	negative	positive	positive

(5) Study of the formation of TP- Δ TP POL- ϵ RNA complex by dividing Terminal Protein (TP) and Reverse transcriptase (RT) regions of POL

As shown in Figure 1A, Terminal Protein (TP) region to which deoxynucleotide is bound, Reverse transcriptase (RT) region having a reverse transcriptase activity, and RNase H region which digests template RNA are present on POL of Hepadnaviridae virus. In the POL competitive assay in (4) above, the translation of the respective regions on POL can not independently be controlled, and the region which corresponds to the substrate of the inhibitory reaction cannot be identified. Therefore, TP region and a region other than TP on POL (Δ TP POL) were translated from the separate RNAs by using the constructs shown in Figure 1 D and E, and the formation of TP- Δ TP POL- ϵ RNA complex was investigated.

In vitro co-translation reaction was carried out using Rabbit reticulocyte Lysate system, nuclease treated (Promega), while the final concentration of RNA derived from the construct of Figure 1E is fixed to be $1.0 \mu\text{g}/\mu\text{l}$ and the final

concentration of RNA derived from the construct of Figure 1D is adjusted to be 1.0 to 0.2 $\mu\text{g}/\mu\text{l}$. To the obtained solution was added ϵ RNA obtained from the construct of Figure 1C at a final concentration of 600 to 20 nM in 1/10 volume, and the mixture was incubated at 30°C for 60 minutes. To 3 μl of the obtained reaction mixture of TP- Δ TP POL- ϵ RNA complex was added 50mM Tris-HCl buffer (pH7.5), 15mM NaCl and 10mM MgCl_2 (each concentration is a final concentration). Further, [α 32P]-dATP (600 Ci/mmol), and cold dTTP, dCTP and dGTP were added at a final concentration of 1.0 μM , the final volume was adjusted to be 5 μl , and the mixture was incubated at 30°C for 80 minutes. After incubation, the reaction solution was subjected to 0.1%SDS-12% polyacrylamide gel electrophoresis according to a conventional method, and the gel was dried and exposed to an imaging plate (Fuji Film). The result is shown in Figure 6. The binding reaction of deoxynucleotide to TP dependent on TP concentration or ϵ RNA concentration was confirmed.

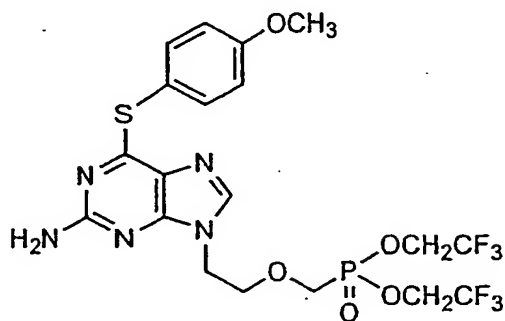
(6) Inhibitory activity of medicament against the formation of TP- Δ TP POL- ϵ RNA complex

Inhibitory activity of medicament against the formation of TP- Δ TP POL- ϵ RNA complex was assayed by performing the aforementioned translation reaction in the presence of a test medicament to bind ϵ RNA at a concentration of 100nM and detecting deoxynucleotide binding reaction and TP. Compound A, Compound B, PMEA and PMEG (9-(2-phosphonmethoxyethyl)guanine) were used as test medicaments. As shown in Figure 7, Compound A, Compound B and PMEG which are 2-aminopurine derivatives inhibited the binding of deoxynucleotide to TP, but PMEA showed no strong inhibition under the same condition.

(7) Evaluation in woodchuck chronic hepatitis models

Each group of 4 woodchucks, which were chronically infected with WHV, were orally administered with 2.5mg/bodyweight kg/day or 10mg/body weight kg/day of Compound C or placebo over two administrations per day for 28 days. The

structure of Compound C is shown below.



As a marker for determining a pharmaceutical effect, a serum WHV DNA level was determined by slot blot method. After the WHV DNA level (pg/ml) was normalized by logarithmic transformation, p value was obtained by Dunnett's test between the mean value in each test group and a placebo control group at various points. The results are shown in the following table 3. After the administration of the medicament was terminated after 28 days, in respect of a 10mg/body weight kg/day administration group, significant decrease of the WHV DNA level was observed until the 112th day, the termination of the observation.

Table: P value vs a placebo control group of the mean log titer of serum WHV DNA level in serum at various points (Dunnnett's test)

Table 3.

	0	1	7	14	21	28	35	42	56	70	84	98	112
Compound C 2.5mg/kg	0.6684	0.9930	0.0046	0.0001	0.0001	0.0003	0.0065	0.0022	0.0324	0.0865	0.7312	0.6562	0.9615
Compound C 10mg/kg	0.9867	0.8793	0.0596	0.0065	0.0003	0.0003	0.0018	0.0003	0.0050	0.1670	0.0982	0.0026	0.0650

Parametric Dunnett two-tailed test

All values were calculated using a SAS package of preclinical test version 4.1.

N=4 all groups from initiation of the test

N=3 10mg of Compound C was administered from the 84th day

From the above results, it was found that compounds A, B and C have a profile different from PME A and its active body, PMEApp, and therefore these compounds are polymerase inhibitors having a novel action mechanism which is different from a competitive action with dNTP. It is considered that the compounds act at the initial stage of the replication of a hepadnaviruses, and inhibit the activation of polymerase by formation of a POL- ϵ RNA complex. The existing anti-viral agent which is competitive with dNTP, has a problem regarding rebound of virus level after drug withdrawal, while the medicament of the present invention, which appears to have a polymerase inactivation action, is expected to have a sustained pharmaceutical effect even after drug withdrawal.

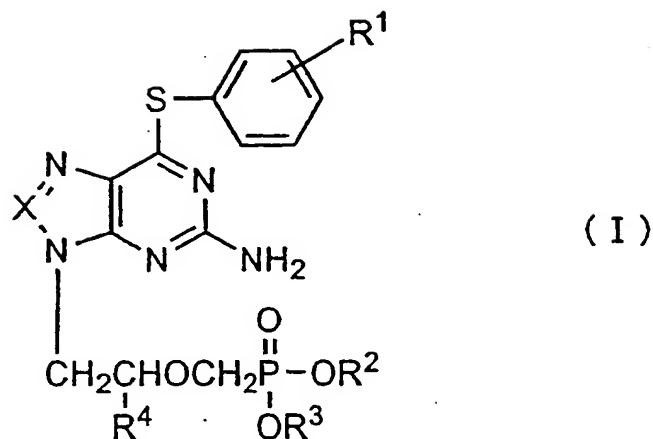
Industrial Applicability

The anti-viral agent of the present invention has an excellent anti-viral activity, a high oral absorbency and high safety to organisms. Moreover, the anti-viral agent of the present invention which has a polymerase inactivation action, has a sustained pharmaceutical effect even after drug withdrawal, that is, after the termination of administration of the medicament.

The present application claims a priority based on JP Application No.2001-262437, which is incorporated herein by reference as a part of the disclosure of the present application.

Claims

1. An anti-viral agent comprising, as an active ingredient, a medicament which inhibits activation of viral polymerase via RNA binding.
2. The anti-viral agent according to claim 1 wherein activation of viral polymerase via RNA binding is activation of viral polymerase via binding of virus RNA and viral polymerase.
3. The anti-viral agent according to claim 1 or 2 wherein RNA is ϵ RNA.
4. The anti-viral agent according to any one of claims 1 to 3, wherein a pharmacological efficacy is sustained after withdrawing the agent.
5. The anti-viral agent according to any one of claims 1 to 4, wherein the virus belongs to Hepadnaviridae.
6. The anti-viral agent according to any one of claims 1 to 5, wherein the virus is a hepatitis B virus.
7. The anti-viral agent according to any one of claims 1 to 6, wherein the medicament is a phosphonate nucleotide compound represented by the following formula (I) or a salt thereof, or a hydrate or solvate thereof:



wherein,

R^1 is a hydroxyl group or a C_1 - C_6 alkoxy group; each of R^2 and R^3 is independently a hydrogen atom, a C_1 - C_{22} alkyl group, an acyloxymethyl group, an acylthioethyl group, or an ethyl group substituted by one or more halogen atoms; R^4 is a hydrogen atom, a C_1 - C_4 alkyl group, a C_1 - C_4 hydroxyalkyl group, or a C_1 - C_4 alkyl group substituted by one or more halogen atoms; and X is CH or a nitrogen atom.

8. The anti-viral agent according to claim 7, wherein R^1 is a hydroxyl group or a methoxy group.

9. The anti-viral agent according to claim 7, wherein each of R^2 and R^3 is independently a hydrogen atom, a C_1 - C_{22} alkyl group, or an ethyl group substituted by one or more halogen atoms.

10. The anti-viral agent according to claim 7, wherein each of R^2 and R^3 is independently a hydrogen atom or a 2,2,2-trifluoroethyl group.

11. The anti-viral agent according to claim 7, wherein R^4 is a hydrogen atom or a methyl group.

12. The anti-viral agent according to claim 7, wherein X is CH.
13. The anti-viral agent according to claim 7, wherein R¹ is a hydroxyl group or a methoxy group, each of R² and R³ is independently a hydrogen atom or a 2,2,2-trifluoroethyl group, R⁴ is a hydrogen atom, and X is CH.
14. The anti-viral agent according to claim 1, wherein the medicament is 2-amino-6-(4-methoxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine bis(2,2,2-trifluoroethyl) ester, 2-amino-6-(4-methoxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine, 2-amino-6-(4-hydroxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine, or 2-amino-6-(4-hydroxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine (2,2,2-trifluoroethyl) ester.
15. A method for screening an anti-viral agent, which comprises a step of evaluating activation of viral polymerase via RNA binding.
16. The method for screening an anti-viral agent according to claim 15, wherein the step of evaluating activation of viral polymerase via RNA binding is that of evaluating the degree of binding of virus RNA and viral polymerase.
17. The method for screening an anti-viral agent according to claim 15 or 16, which comprises steps of adding a test medicament to viral polymerase, adding virus RNA, and evaluating the degree of binding of virus RNA and viral polymerase.
18. The method for screening an anti-viral agent according to any one of claims 15 to 17, which comprises steps of adding a test medicament to viral polymerase, adding virus RNA, and judging that the test medicament is an anti-viral agent when the test medicament inhibits the binding of virus RNA and viral polymerase.
19. The method for screening an anti-viral agent according to any one of claims 15 to 18, wherein RNA is ϵ RNA.
20. The method for screening an anti-viral agent according to any one of claims 15 to 19, wherein the virus belongs to Hepadnaviridae.
21. The method for screening an anti-viral agent according to any one of claims 15 to 20, wherein the virus is a hepatitis B virus.
22. An anti-viral agent obtained by the method for screening an anti-viral agent

according to any one of claims 15 to 21.

23. An anti-viral agent obtained by the steps of conducting the method for screening an anti-viral agent according to any one of claims 15 to 21 to obtain an anti-viral substance, producing the thus obtained anti-viral substance by chemical synthesis, and mixing the anti-viral substance with a pharmaceutically acceptable carrier.

Figure 1

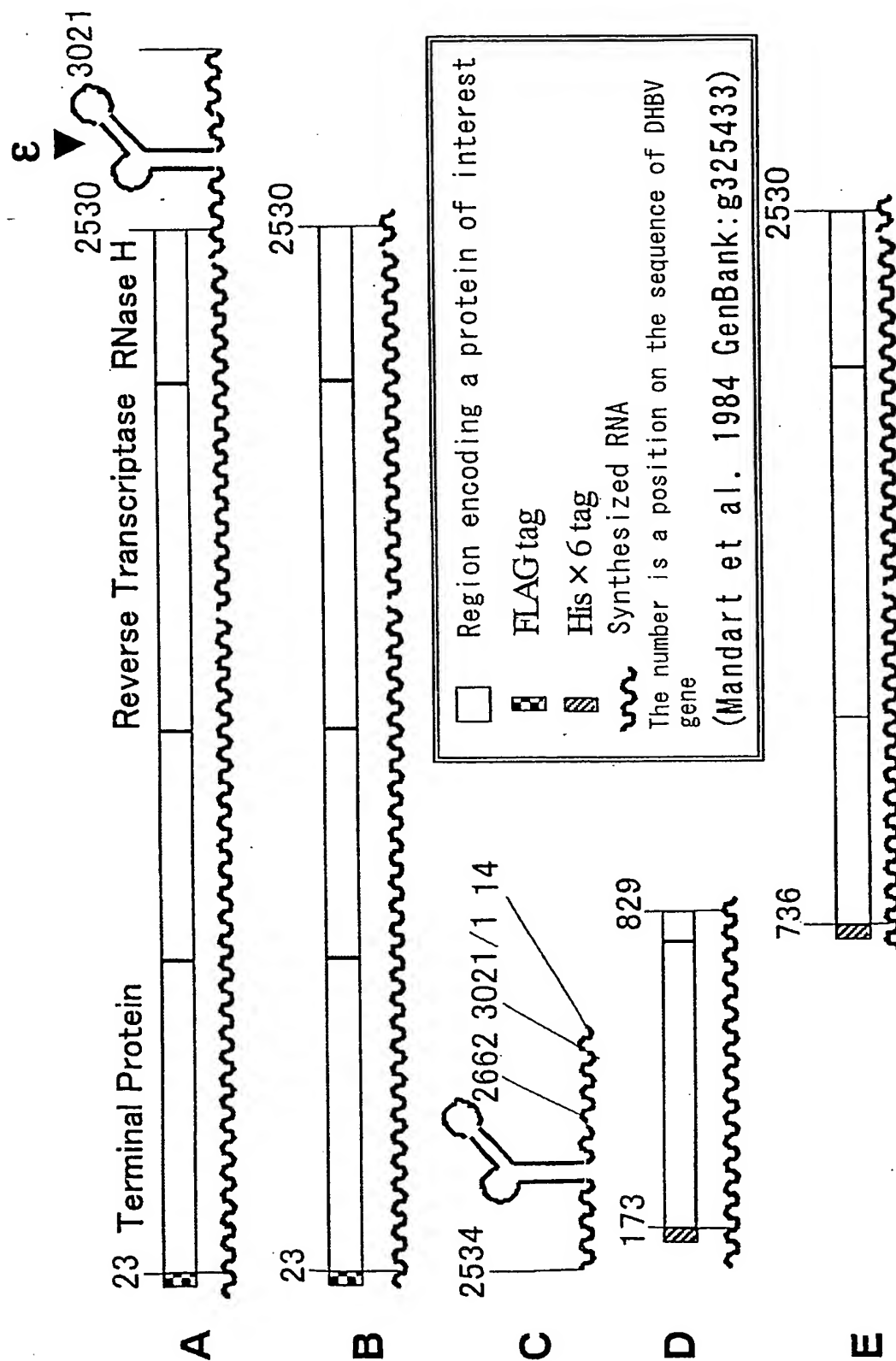
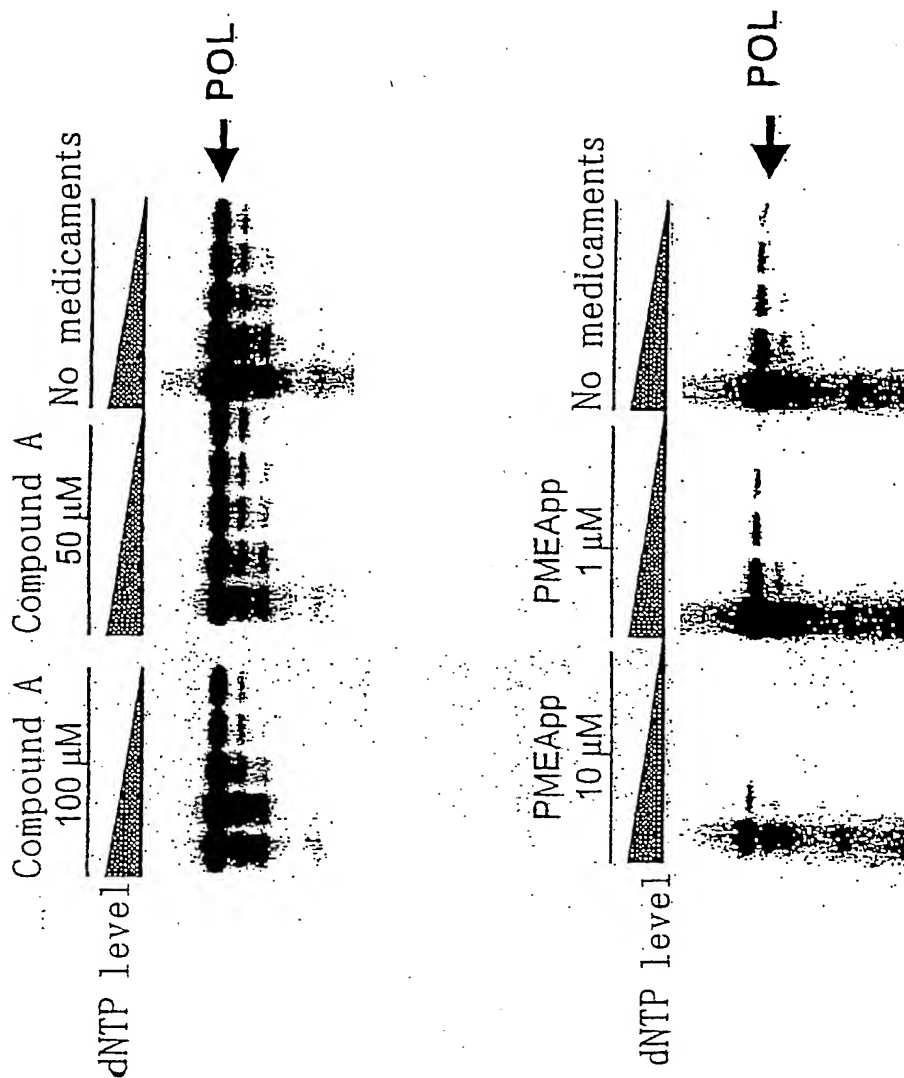
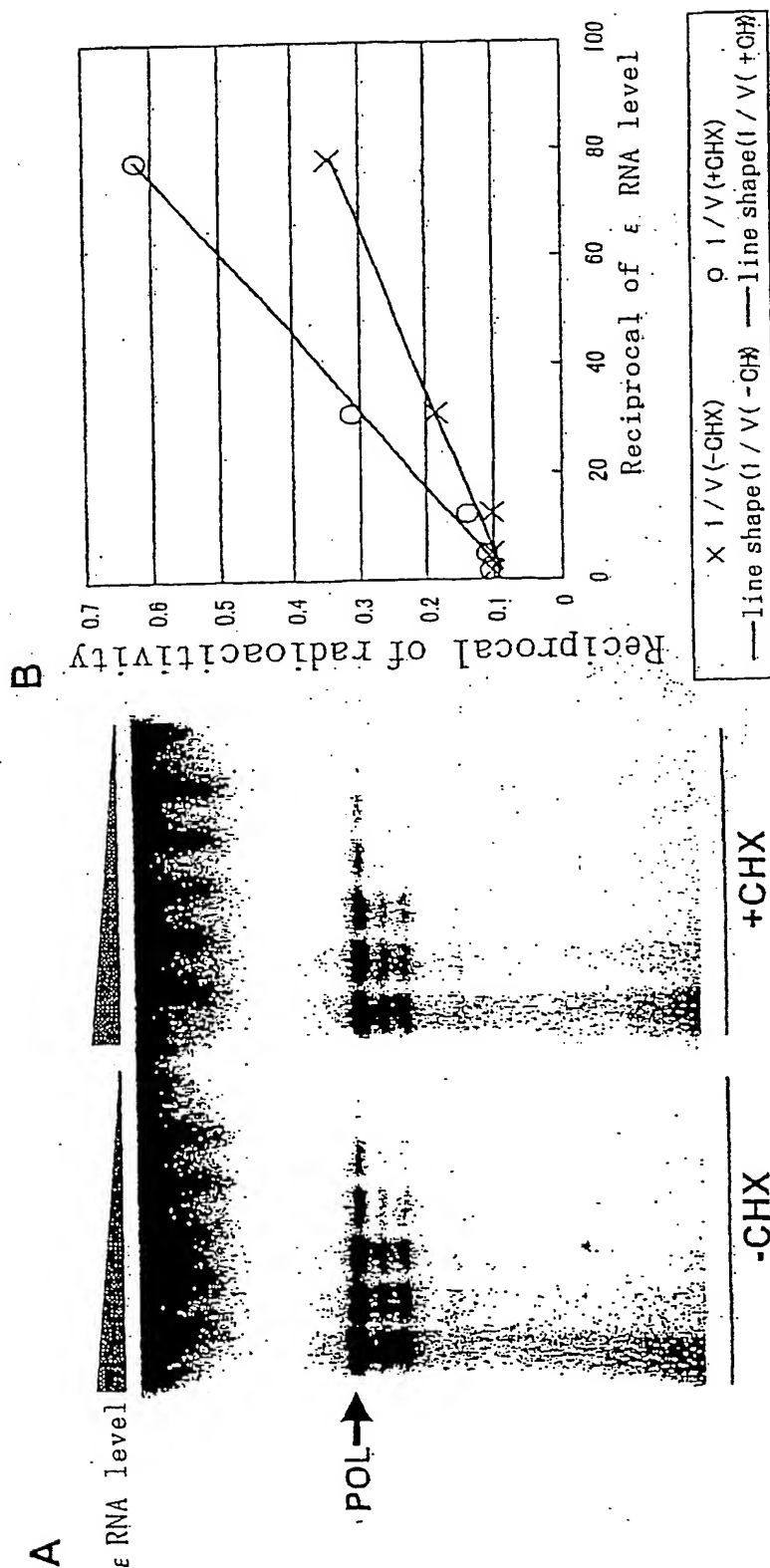


Figure 2



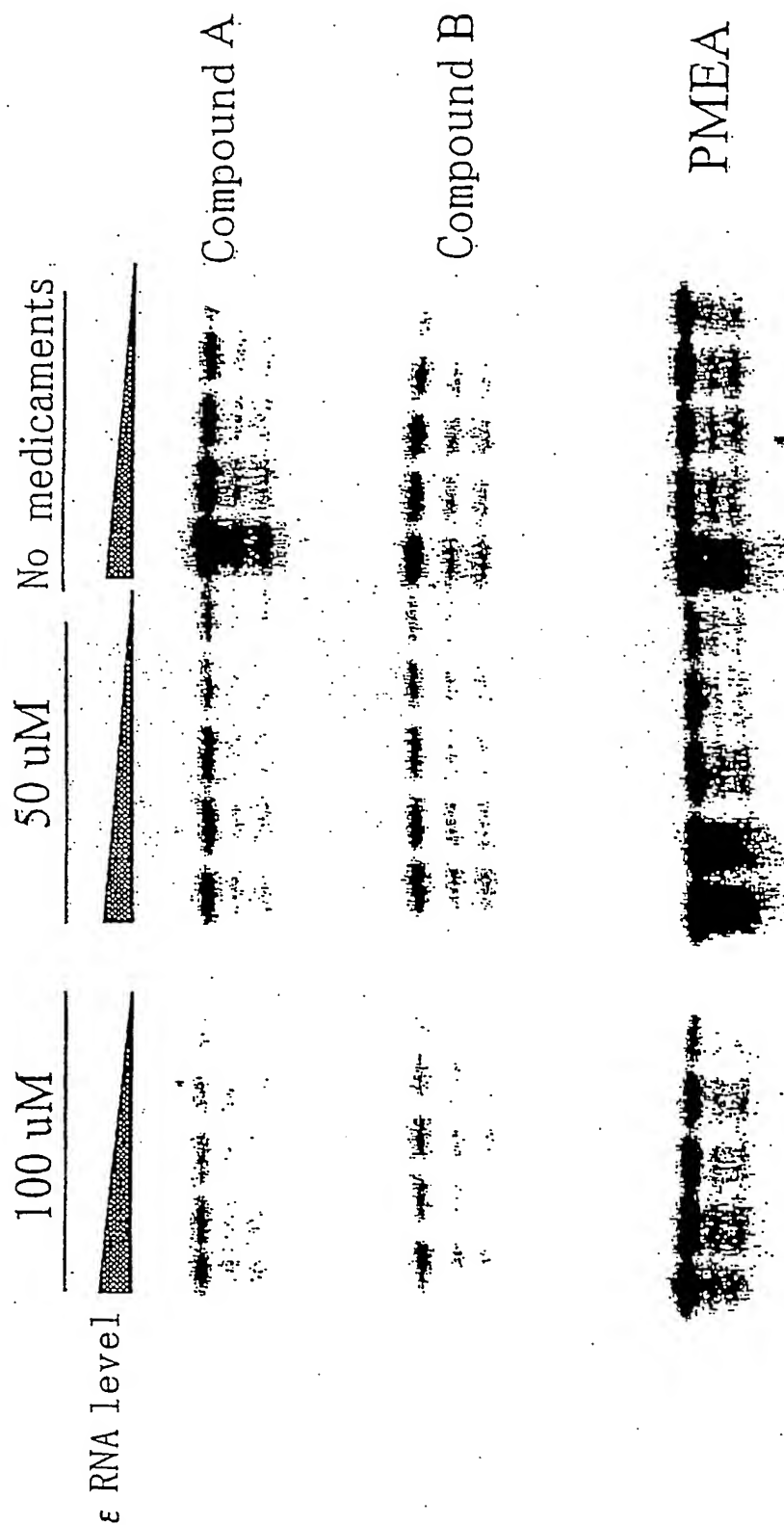
Test examples performed by conventional method

Figure 3



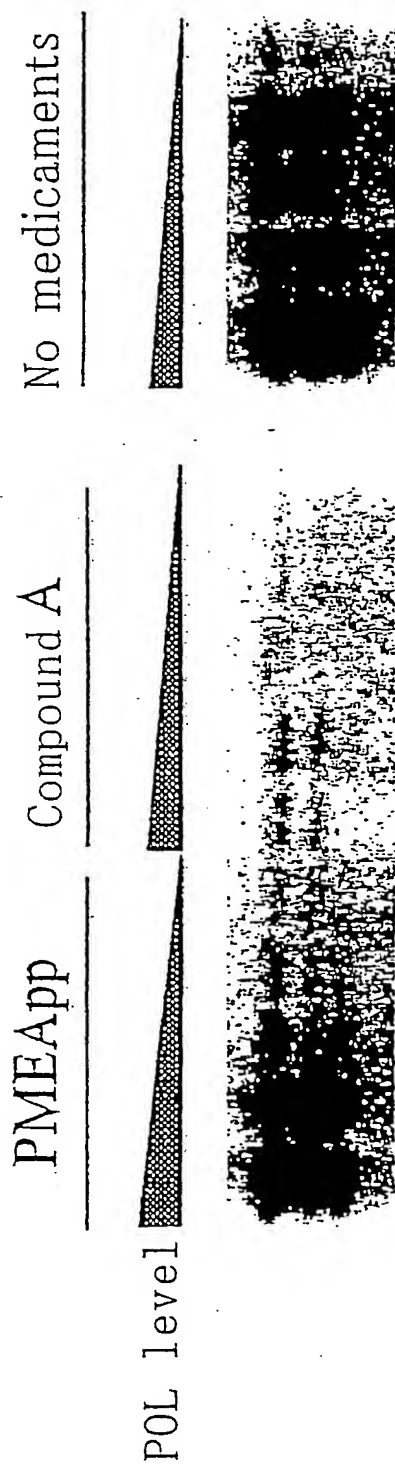
ϵ RNA level dependency in improved method
A: Analysis example B: Double reciprocal plot
+CHX: treated with cycloheximide
-CHX: untreated with cycloheximide

Figure 4



ϵ RNA competition test example performed by improved method

Figure 5



POL level dependency analysis example performed
by improved method

Figure 6

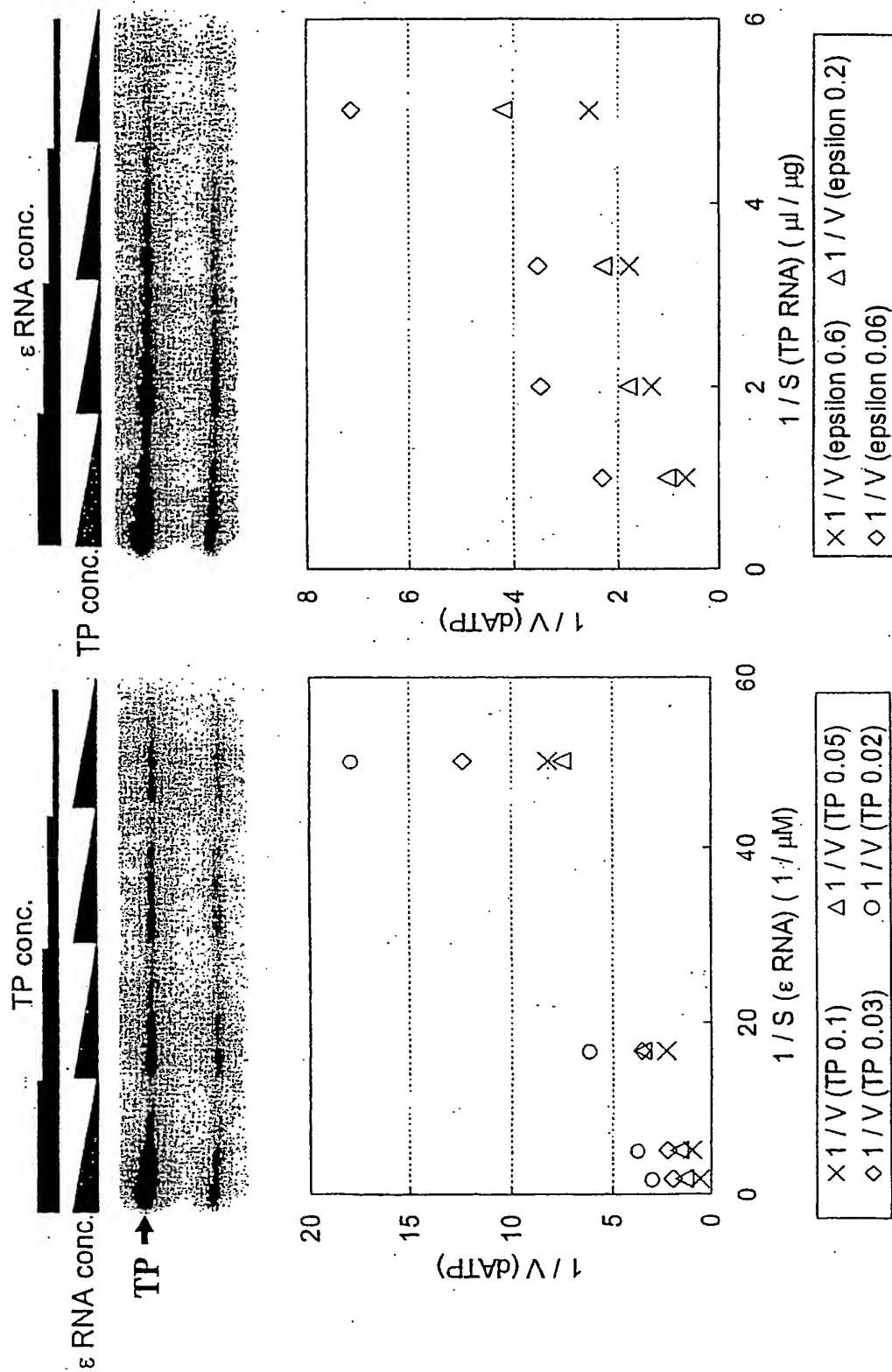
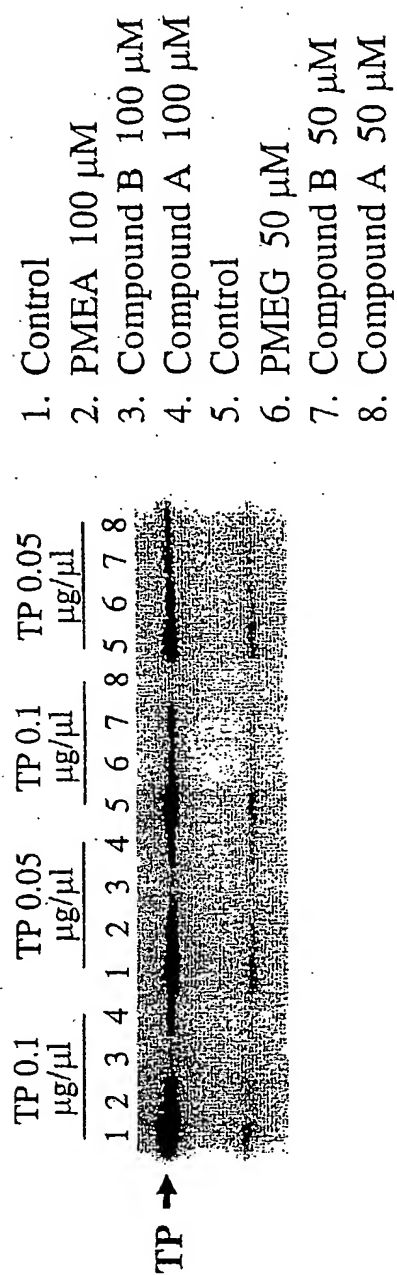


Figure 7



A. CLASSIFICATION OF SUBJECT MATTER IPC 7 A61K31/683		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC 7 A61K		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used) EPO-Internal, PAJ, MEDLINE, BIOSIS, CHEM ABS Data, WPI Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 785 208 A (MITSUBISHI CHEM CORP) 23 July 1997 (1997-07-23) examples 4,248 page 52; example 1 page 46, line 43	1-14, 22, 23
X	WO 94 03467 A (REGA STICHTING ;BALZARINI JAN MARIE RENE (BE); DECLERCO ERIK DESIR) 17 February 1994 (1994-02-17) page 7, line 20 page 7, line 35 page 22, line 1 - line 3 examples 1-49 example 50 table I	1-6, 22, 23
-/--		
<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex.		
* Special categories of cited documents : *A* document defining the general state of the art which is not considered to be of particular relevance *E* earlier document but published on or after the international filing date *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) *O* document referring to an oral disclosure, use, exhibition or other means *P* document published prior to the international filing date but later than the priority date claimed *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. *&* document member of the same patent family		
Date of the actual completion of the international search		Date of mailing of the international search report
9 January 2003		23/01/2003
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016		Authorized officer Giacobbe, S

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 33200 A (CLERCQ ERIK DESIRE ALICE DE ;REGA STICHTING (BE); HOLY ANTONIN (CZ) 24 October 1996 (1996-10-24) page 3, line 19 -page 6, line 15 page 17, line 34 page 18, line 10 ---	1-6,22, 23
X	EP 0 618 214 A (MERRELL DOW PHARMA) 5 October 1994 (1994-10-05) page 2, line 14 -page 3, line 30 claims 13,14 ---	1-4,22, 23
X	EP 0 632 048 A (MITSUBISHI CHEM IND) 4 January 1995 (1995-01-04) claims 1-14 ---	1-6,22, 23
X	EP 0 832 896 A (MITSUBISHI CHEM CORP) 1 April 1998 (1998-04-01) abstract claims 1-13 page 33; example 1 ---	1-6,22, 23
X	EP 0 919 562 A (MITSUBISHI CHEM CORP) 2 June 1999 (1999-06-02) abstract table 1 page 47; example 1 claims 1-5 ---	1-6,22, 23
X	US 6 037 335 A (TAKASHIMA HIDEAKI ET AL) 14 March 2000 (2000-03-14) abstract examples 1-26 column 51, line 26 - line 62 table 8 claims 1-7 ---	1-6,22, 23
Y	TAVIS JOHN E ET AL: "Evidence for activation of the hepatitis B virus polymerase by binding of its RNA template." JOURNAL OF VIROLOGY, vol. 70, no. 9, 1996, pages 5741-5750, XP002221466 ISSN: 0022-538X abstract ---	15-21

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>HU JIANMING ET AL: "Hepadnavirus assembly and reverse transcription require a multi-component chaperone complex which is incorporated into nucleocapsids." EMBO (EUROPEAN MOLECULAR BIOLOGY ORGANIZATION) JOURNAL, vol. 16, no. 1, 1997, pages 59-68, XP002221467 ISSN: 0261-4189 abstract</p>	15-21
Y	<p>TAVIS JOHN E ET AL: "The duck hepatitis B virus polymerase is activated by its RNA packaging signal, epsilon." JOURNAL OF VIROLOGY, vol. 72, no. 7, July 1998 (1998-07), pages 5789-5796, XP002221468 ISSN: 0022-538X abstract</p>	15-21
Y	<p>STASCHKE K A ET AL: "Drug discovery and development of antiviral agents for the treatment of chronic hepatitis B virus infection." PROGRESS IN DRUG RESEARCH. FORTSCHRITTE DER ARZNEIMITTELFORSCHUNG. PROGRES DES RECHERCHES PHARMACEUTIQUES. SWITZERLAND 2001, vol. Spec No, 2001, pages 111-183, XP001121546 ISSN: 0071-786X page 119, paragraph 2 -page 121, paragraph 2</p>	15-21
Y	<p>STASCHKE K A ET AL: "Priming of duck hepatitis B virus reverse transcription in vitro: premature termination of primer DNA induced by the 5'-triphosphate of fialuridine." JOURNAL OF VIROLOGY. UNITED STATES DEC 1994, vol. 68, no. 12, December 1994 (1994-12), pages 8265-8269, XP009001414 ISSN: 0022-538X cited in the application abstract</p>	15-21

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>COLACINO J M ET AL: "The identification and development of antiviral agents for the treatment of chronic hepatitis B virus infection."</p> <p>PROGRESS IN DRUG RESEARCH. FORTSCHRITTE DER ARZNEIMITTELFORSCHUNG. PROGRES DES RECHERCHES PHARMACEUTIQUES. SWITZERLAND 1998, vol. 50, 1998, pages 259-322, XP000885900</p> <p>ISSN: 0071-786X</p> <p>page 270, paragraph 5</p> <p>----</p>	15-21
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